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(54) Title: A NOVEL ROOT SPECIFIC PROMOTER DRIVING THE EXPRESSION OF A NOVEL LRR RECEPTOR-LIKE KINASE

(57) Abstract: The present invention relates to the field of plant molecular biology, more particularly to the root-specific gene expression in plants. The invention provides nucleic acids for a novel transcriptional regulatory root-specific promoter and nucleic acid and protein sequences coding for a new LRR receptor-kinase protein, further specified as a root clavata 1 homolog (RCH1). Further provided are compositions comprising nucleic acids, polypeptides, antibodies and vectors. The invention further provides for methods for modifying cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology comprising the modification of expression in particular cells, tissues or organs of a plant of the novel LRR receptor-like kinase or comprising the expressing of a gene of interest under the control of the novel transcriptional regulatory root-specific promoter. Further are provided compounds interacting with the new polypeptides for use as herbicides or growth regulators.

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## A NOVEL ROOT SPECIFIC PROMOTER DRIVING THE EXPRESSION OF A NOVEL LRR RECEPTOR-LIKE KINASE

### FIELD OF THE INVENTION

5 The present invention relates to the field of plant molecular biology, more particularly to the root-specific gene expression in plants. The isolation of a root specific operon, comprising a transcriptional regulatory promoter that contributes to root-specific gene expression and its operably linked gene encoding a novel Leucin Rich Repeat (LRR) receptor-like kinase, is disclosed. Said transcriptional regulatory promoter may be used  
10 for driving root-specific expression of at least one gene of interest in a transgenic plant and said encoded LRR receptor-like kinase gene may be used to alter the features and/or to confer a selective advantage to transgenic plants.

### BACKGROUND TO THE INVENTION

#### 15 Plant promoters

Initiation of transcription is generally understood to be the predominant controlling factor in determining expression of a gene. The transcriptional control elements, which may interact with DNA binding proteins, are generally embedded in the sequence 5'-flanking or upstream of the transcribed gene. These DNA sequence elements promote the  
20 formation of transcriptional regulatory complexes that either activate or repress the expression of the gene 3' downstream of the promoter.

The sequences of the promoter vary in length and base pair composition from gene to gene. Within the sequence of the promoter, many regulatory sequence elements can be embedded that control the promoter activity. These specific elements, called DNA-  
25 boxes, contribute to the promoter a defined feature or a defined activation pattern. Many of these boxes are recognition sites to which regulatory transcription factors can bind and are part of a tight control mechanism of the promoter. Numerous plant promoter DNA boxes have been described, such as tissue-specific boxes (Chaubet et al. 1996), pyrimidin boxes (Gubler & Jacobsen 1992), which influence the level of expression or G-  
30 boxes (Dolferus et al. 1994) which reduce promoter activity by cold or dehydration.

Constitutive promoters are promoters that drive the expression of genes in a continuous manner, whereas ubiquitous promoters drive the expression of the gene throughout the entire plant.

Expression of heterologous DNA sequences in a host is dependent upon the presence of an operably linked promoter that is also present in that host, whereby the choice of promoter will determine when, where or how strong the heterologous DNA sequence will be expressed in the host organism. Frequently those skilled in the art desire to limit the expression of the transfected DNA sequence to a certain time-period (e.g. to a particular phase of plant development), to a defined tissue of the host (e.g. leaves), or to a certain expression level. Controlling the expression of the heterologous genes in transgenic plants is considered to provide several advantages to the plant over ubiquitous and constitutive expression. There is a broad need in many industrial applications for transcriptional control elements capable of driving tissue specific gene-expression in plants. It is therefore important that such control elements are continuously provided and that new control elements with their specific transcriptional features are isolated and characterized.

### **Promoter regulation**

Promoters from both monocots and dicots that have a root root-preferred expression pattern have already been described (Fowler, Bernhardt, & Tierney 1999;Hwang & Goodman 1995;Lagarde et al. 1996;Okada et al. 2000;Okumura et al. 1994;Pysh et al. 1999;Schneider et al. 1998;Vidmar et al. 2000;Wanapu & Shinmyo 1996;Winicov 2000)WO9007001(Baszczyński et al. 2000;Borg et al. 1997;Masuda, Sakuta, & Satoh 1999;Webb et al. 2000) (de Pater & Schilperoort 1992;Ely, Evans, & Schuch 2000;Goddemeier, Wulff, & Feix 1998;Kikuchi et al. 1999;Suzuki, Fowler, & Tierney 1993;Unbe et al. 1998;Woo & Hawes 1997;Xu et al. 1995) (Yamamoto et al. 1991) (Gritch et al. 1997;Keller & Lamb 1989;Lauter 1996;Olson, Oetiker, & Yang 1995;van der Zaal et al. 1991). Many of them are root-preferred rather than root-specific and are expressed at low levels in tissues other than the root. These promoters are not suitable for industrial applications where it is of the utmost importance that the heterologous product is expressed in the root only. As an example, ectopic expression of cell cycle control elements to obtain growth arrest in some parts of the root (e.g. lateral roots),

should be strictly regulated to that tissue in order not to interfere with the overall growth of the plant. In contrast, other promoters are strictly and exclusively confined to root-specific expression and can therefore be valuable for some applications demanding tight control of the expression. In this group of root-specific promoter subgroups can be distinguished based on the root-tissue they are expressed in. The HRGPnt3 gene of tobacco for instance is only expressed in the pericycle and the endodermis of the root, more specifically in the discrete subset of cells, involved in the initiation of the lateral roots (Keller et al. 1989). This makes the promoter of HRGPnt3 highly specific. Another example is the XSP30 sequence, which is only activated in the xylem of the root.

For persons skilled in the art it is of importance to have at their disposal a wide variety of root-specific promoters with different root tissue-specificity from which they can choose to confer a specific feature to the transgenic plant.

Many of these root-preferred promoters are not only limited to tissue specific expression, but are also subjected to a tight control mechanism deciding when the promoter should be active. This decision depends on the function and the effects of the gene product that is switched on/off by the related promoter. Some gene products are switched on/off at a certain developmental stage of the plant, at a certain moment of the cell cycle, in stress situations originating from weather factors, upon injury caused by pathogens or upon a change in the environmental resources. A typical example is the ARSK1 gene of *Arabidopsis thaliana*, that is activated when the roots are exposed to air during growth (dehydration) or by treatment of roots with abscisic acid or salt (Hwang et al. 1995).

However, for many industrial and agronomic applications, where the aim is to confer a special feature at any time of the developmental, environmental or physical state of the plant, a constitutive promoter will be needed. For example when the gene product of the transgene is to be isolated and purified from the plant for commercial purposes.

It is important for the persons skilled in the art to have at their disposal a wide variety of promoters with different time-control features from which they can chose to confer a specific feature to the transgenic plant.

### **Plant root development**

Root development is an essential determinant of plant growth and crop yield since the root is the main channel to extract nutrients from the environment. Any changes in the



root that contribute to more functional organ tissue will result in higher crop yield. Also any regulatory element that is involved in the root development and whose control is subjected to developmental events can contribute better yield properties to a transgenic plant.

5 Cells of the shoot and root meristems are mitotically active and they extend the undifferentiated tissue pattern of the embryo as a pool of cells from which cells can differentiate into several tissues of the adult plant. This process continues throughout the life span of the plant. It is clear that the root meristem plays a pivotal role in the formation of strong roots and therefor also in affecting the growth and crop yield.

10 In root meristem, the emerging picture is that the integrity of the root meristem is kept by balancing cell proliferation and cell differentiation, and that differentiation-inhibiting signals originating from the quiescent center are involved (van den Berg et al. 1997).

Inhibition of differentiation only occurs in cells that contact the quiescent center, which reveals that short-range or contact-dependent signals are involved in this process.

15 However which compounds play a role in this process and whether positional cues guiding root cell fate are targeted via plasmodesmata or via the cell wall remains unknown (van den Berg et al. 1997).

In the shoot meristem more progress has been made in characterizing molecular components involved in meristem maintenance. *Clavata* (*clv*) mutants accumulate massive pools of undifferentiated cells in the central zone of shoot and floral meristem. The *CLAVATA* genes show a shoot and floral meristem specific expression pattern. Root meristems of *clv*-mutants are unaffected (Clark, Running, & Meyerowitz 1993). The *CLAVATA* loci (*CLV1*, *CLV2* and *CLV3*) appear to promote the transition toward differentiation of cells at the shoot and floral meristems, and/or to restrict the proliferation of cells at the center of these meristems (Clark et al. 1993). *CLV1* acts with *CLV3* and *WUSCHEL* (*WUS*) and *SHOOT MERISTEMLESS* (*STM*) to maintain the integrity of the central zone which acts as a reservoir of stem cells. A model for stem-cell maintenance in the root meristem and in the shoot meristem is depicted in Figure 1.

### 30 **CLV1 homologs and CLV1-redundancy**

The *CLAVATA1* gene resides on the *Arabidopsis thaliana* chromosome 1. *Clv1* mRNA is found in the L3 tunica cell layer of the central zone and encodes a protein with leucine-rich repeats (LRR, of a type found in a number of proteins, notably the products of

several plant-pathogen resistance genes), a putative serin/threonin kinase domain and a predicted extracellular receptor domain. Expression of the *clv1* gene is initiated in the heart stage of the embryo, when cotyledonary primordia are apparent, and is independent of the *STM* (*SHOOT MERISTEMLESS*) activity (Long & Barton 1998).

5 *CLAVATA1* is a leucine-rich receptor-like kinase. The *Arabidopsis thaliana* genome project has so far identified over 50 LRR transmembrane receptor serine/threonine kinases. It can be estimated that the total *Arabidopsis thaliana* genome contains in the order of 100 family members. Other members of the *CLAVATA* family are *ERECTA* (*ER*) (Tom et al. 1996), *BRASSINOLIDE INSENSITIVE* (*BR*) (Li & Chory 1997) and *HAESA* (*HAE*) (Jinn, Stone, & Walker 2000), genes which have a function in plant development. Analogues of the *Arabidopsis thaliana* *CLAVATA* genes were also found in soybean (*Glycine max*). They were isolated from the wild type and the fasciating mutant of soybean and were designated GmCLVA1 and GmCLV1B (Yamamoto, Karakaya, & Knap 2000).

15 It is the aim of the present invention to isolate novel promoters and genes which are useful for altering features of transgenic plants and thus confer selective advantage to transgenic plants.

All the aims of the present invention have been met by the formulation of the following preferred embodiments of the current invention.

20

## SUMMARY OF THE INVENTION

In the present invention a DNA operon was isolated from *Arabidopsis thaliana*, which comprises a novel root-specific promoter, driving a gene encoding a Leucine Rich Repeat (LRR) receptor-like kinase. Unexpectedly, the inventors discovered that the new  
25 operon was specifically expressed in roots and more surprisingly in the meristem of the main and lateral roots, in the main and lateral root vascular tissue and in the lateral root primordia.

The surprisingly specific expression pattern, as well as the very strong expression level, makes the newly found promoter a very attractive and selective tool to drive root-specific  
30 expression of any gene of interest. The ability to target meristem cells of the root makes this promoter clearly distinct from the root-specific promoters presently known in the art. Another surprising aspect of the invention is the identification of the new LRR receptor-

like kinase gene, which is the first gene identified in roots showing homology with a *clavata*-gene. This gene is specifically expressed in roots in a specific developmental stage of the root cells (namely in the meristematic phase) and can therefore contribute agronomic interesting features to a transgenic plant when transfected herein. Therefore the root-specific promoter is further denominated as ROOT *CLAVATA* HOMOLOG 1 promoter (RCH1prom) and the LRR receptor-like kinase as RCH1.

Accordingly, the invention embodies an isolated DNA sequence with nucleotide sequence as given in SEQ ID NO 1 (Figure 5) comprising a novel root specific regulatory promoter sequence with nucleotide sequence as given in SEQ ID NO 2 (Figure 6) or SEQ ID NO 18 (Figure 13), and an isolated DNA sequence with nucleotide sequence as given in SEQ ID NO 3 (Figure 8), encoding a novel ROOT *CLAVATA* HOMOLOG 1 gene, with amino acid sequence given in SEQ ID NO 4 or SEQ ID NO 19 (Figure 9).

Furthermore the invention embodies a method for modifying cell fate and/or plant development and/or plant morphology and/or biochemistry and/or physiology comprising the modification of expression in particular cells of the root of a particular transgene operably linked to the RCH1 promoter sequence of the present invention. Provided in the current invention are methods to effect expression of any gene of interest in particular cells of the plant root. The present invention also relates to a method to effect expression of a ROOT *CLAVATA* HOMOLOG 1 protein or a homologue or a derivative thereof in a plant cell, tissue or organ. The present invention clearly extends to any plant produced by the inventive methods described herein.

## DETAILED DESCRIPTION OF THE INVENTION

One of the technical problems underlying the present invention is to provide regulatory elements that are active in the root only and that show no expression in other tissues. Additional to the highly selective and tissue-specific regulation of a promoter, other features such as very strong activity, continuous activity, and termination of activity at a certain developmental stage of the cells, would contribute an unique combination of regulatory elements to specifically regulate the expression of a gene of interest in the roots of a transgenic plant, thereby providing a means for modifying cell fate and/or plant development and/or plant morphology and/or biochemistry and/or physiology. Also the isolation of root-specific DNA sequences encoding proteins that could play a role in root

development is envisaged for use in modification of cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology.

In a first embodiment, the present invention relates to an isolated nucleic acid comprising a transcriptional regulatory root promoter sequence and/or a nucleic acid sequence  
5 encoding a novel LRR receptor-like kinase (protein) or an immunologically active and/or functional fragment of such a regulatory element or protein (kinase) selected from the group consisting of:

- (a) nucleic acid comprising at least part of the DNA sequence as given in any of SEQ ID NOs 1, 2, 3 or 18, or the complement thereof,
- 10 (b) nucleic acid comprising the RNA sequences corresponding to at least part of any of SEQ ID NOs 1, 2, 3 or 18, or the complement thereof,
- (c) nucleic acid specifically hybridizing, preferably under stringent conditions, with the nucleotide sequence as defined in (a) or (b),
- (d) nucleic acid encoding a protein comprising the amino acid sequence as given in  
15 SEQ ID NO 4, SEQ ID NO 5 or SEQ ID NO 19,
- (e) nucleic acid which is degenerated as a result of the genetic code to a nucleotide sequence encoding a protein as given in SEQ ID NO 4, SEQ ID NO 19 or to a nucleotide sequence as defined in (a) to (d),
- (f) nucleic acid which is diverging due to the differences in codon usage between  
20 the organisms to a nucleotide sequence encoding a protein as given in SEQ ID NO 4, SEQ ID NO 19 or to a nucleic acid sequence as defined in (a) to (d),
- (g) nucleic acid encoding a protein as given in SEQ ID NO 4, SEQ ID NO 19 or as defined in (a) to (d), which is diverging due to the differences between alleles encoding a protein
- 25 (h) nucleic acid encoding a fragment of a protein encoded by a DNA sequence as given in SEQ ID NO 1 or SEQ ID NO 3 or encoding a fragment of a protein encoded by a nucleic acid as defined in any one of (a) to (g), wherein said fragment comprises the sequence as represented in SEQ ID NO 5, and,
- (k) nucleic acid encoding a protein as defined in SEQ ID NO 4, SEQ ID NO 19 or  
30 nucleic acid as defined in any one of (a) to (h), said nucleic acid interrupted by intervening DNA sequences.

The sequence as depicted in SEQ ID NO 1 is part of a sequence deposited under the Genbank Accession number AB017061, which represents the entire *Arabidopsis*

*thaliana* chromosome 5. The nucleic acid of the invention is not the nucleic acid with the sequence as deposited under the Genbank Accession number AB017061 (Figure 14).

Furthermore, in a preferred embodiment, the present invention relates to an isolated nucleic acid comprising a novel root-specific promoter sequence, termed RCH1prom, isolated from *Arabidopsis thaliana* and consisting of the nucleic acid as represented in SEQ ID NO 2 or SEQ ID NO 18, or a functional part of said sequence, which is able to regulate gene expression in a root specific manner as SEQ ID NO 2 or SEQ ID NO 18 itself. More specifically, said isolated DNA sequence provides a novel type of a root-specific promoter, which differs from other known root-specific promoters in the fact that it is unexpectedly active in only distinct cells of the root, namely the meristem of the main and the lateral root, as well as in the vascular tissue of the main and lateral root and in the lateral root primordia.

The isolation of the root specific promoter RCH1 of the present invention is described in Example 1.

Another aspect of the invention is the isolation and sequencing of a root-specific Leucin Rich Repeat (LRR) receptor-like kinase gene from root tissue of *Arabidopsis thaliana* and is also described in Example 1. The nucleotide sequence is listed in the current specification as SEQ ID NO 3 and illustrated in Figure 8. This sequence is present as part of the sequence deposited under the GenBank Accession number AB017061, which represents the entire *Arabidopsis thaliana* chromosome 5.

The novelty of the RCH1 promoter sequence relative to known root-specific promoters is substantiated by its unique expression pattern in plant roots. The analysis of the RCH1 expression patterns was done using a RCH1prom-GUS hybrid operon that was transformed to *Arabidopsis thaliana* as outlined in Example 2. Analogous experiments with the RCH1prom-GUS hybrid operon were performed to establish the expression pattern of RCH1 in embryo's (Example 8 and Figure 15).

The RCH1 promoter is active in the meristem of the main and the lateral roots, as well as in the vascular tissue of the main and the lateral root and in the lateral root primordia.

The promoter of the present invention is the first known promoter that regulates a *CLAVATA* HOMOLOG gene in roots, and is clearly distinct from other described *CLAVATA*-operons which are all active in the shoot and floral meristem. The present data thus clearly distinguish RCH1prom from other plant root-specific promoters, which

are not expressed in the meristem of the main and lateral root, in the vascular tissue of the main and lateral root and in the lateral root primordia.

From the results obtained by the RCH1::GFP expression studies in root it was even more clear that the RCH1 promoter is strongly active in the endodermis, cortex, epidermis, lateral root cap, in short in the "division zone". The RCH1 promoter is also active but in a lower manner, in the quiescent center and in the vascular tissue.

A further characteristic of the RCH1 promoter is its very high expression level. Based on the incubation time of the RCH1prom-GUS hybrid operon transformed plant with the GUS substrate (1 hour) and the very high output signal in the root of that plant (Example 2, Figure 7D), it is clear for persons skilled in the art that the RCH1 promoter is very strong.

A further characteristic of the present invention is that the activity of the RCH1 promoter in the meristem cells stops when these cells start to differentiate and to elongate to become cells of specified root tissue other than root vascular tissue. Surprisingly the promoter is activated again in cells of the pericycle that form lateral root primordia. This expression is extended in the meristem of the lateral root, but again, the cells that differentiate into lateral root tissue other than vascular tissue do not retain RCH1 promoter activity.

Functional parts of the promoter of the invention will have at least one of these characteristics.

According to a further embodiment, the present invention relates to an isolated nucleic acid comprising a transcriptional regulatory root promoter comprising at least part of a sequence as given in SEQ ID NO 1, 2 or 18.

According to another embodiment, the present invention relates to an isolated nucleic acid as represented in SEQ ID NO 2 or 18 wherein said transcriptional regulatory root promoter is operably linked to said nucleic acid sequence encoding a novel LRR receptor-like kinase or to an immunologically active and/or functional fragment thereof.

According to the invention, distinctive fragments of the newly identified promoter, which contribute specific features to said promoter, can be fused to another nucleic acid sequence (e.g. a promoter) in order to alter the sequential and/or regulatory features of the latter.

The invention thus relates to an isolated nucleic acid having transcriptional regulatory root specific, root-meristem specific or root-vascular-tissue specific promoter activity

comprising at least part of the DNA sequence as given in any of SEQ ID 1, 2 or 18 and a second transcriptional regulatory sequence.

In one embodiment, said second transcriptional regulatory sequence is for instance an inducible box or sequence, which is placed within the RCH1 promoter, wherein the  
5 function of the RCH1 promoter is not hampered but instead can be regulated, for instance by induction. In the latter embodiment, the RCH1 promoter is not used to confer root-specificity to other promoters, but a second sequence is used to confer an additional regulatory feature to the RCH1 promoter.

In another embodiment said second transcriptional regulatory sequence is a promoter  
10 sequence not normally exhibiting root-specificity, thereby resulting in a hybrid promoter with root-specificity.

Accordingly, another embodiment of the present invention is to confer root-specificity, root-meristem-specificity, and root-vascular-tissue-specificity and/or root abundant gene expression to other promoter sequences. This can be achieved by fusing elements of  
15 the here-disclosed RCH1 promoter to the promoter sequence of interest preferably the widely used ubiquitin promoter. Such modifications can be achieved by routine experimentation by those skilled in the art.

It should be further understood that the invention preferably relates to any of the isolated nucleic acids as described above which is DNA, cDNA, genomic DNA, synthetic DNA or  
20 RNA wherein T is replaced by U.

The invention also relates to a nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with any of the nucleic acids of the invention. The invention also relates to a nucleic acid molecule of at least 15 nucleotides in length specifically amplifying a nucleic acid of the invention.

Further, the invention relates to a vector comprising a nucleic acid of the invention. Said  
25 vector may be an expression vector wherein the (said) nucleic acid sequence encoding a novel LRR receptor-like kinase or an immunologically active and/or functional fragment thereof, is operably linked to one or more control sequences allowing the expression in prokaryotic and/or eukaryotic host cells.

The invention also relates to a vector comprising at least part of a nucleic acid according  
30 to the invention and wherein a transcriptional regulatory root promoter of the invention or functional part(s) thereof, is operably linked to one or more genes of interest.

The invention also relates to a host cell containing a nucleic acid molecule of the invention or a vector of the invention wherein said nucleic acid or vector has been introduced by transformation, transfection or infection. Preferred host cells that are part of the invention are bacterial, insect, fungal, plant or animal cells.

- 5 Therefore, in a preferred embodiment, the invention relates to an isolated nucleic acid as represented in SEQ ID NO 3 encoding a LRR receptor-like kinase characterized by an amino acid sequence as given in SEQ ID NO 4 or 19. More specifically, said isolated nucleic acid encodes a plant ROOT CLAVATA HOMOLOG 1 (RCH1) of a novel type which is unexpectedly expressed in roots. Said novel plant RCH1 shows significant  
10 homology with CLAVATA 1, a protein involved in the maintenance of floral and shoot meristem cells.

An initial comparative amino acid sequence homology search using the BLASTP 2.0.8 software (Altschul et al. 1997) revealed that most significant alignments of RCH1 protein are produced with receptor-like protein kinases, more specifically with leucine rich  
15 receptor-like kinases such as CLAVATA1 (CLV1). Furthermore, the most homologous protein with a known function of this list, is the CLAVATA 1 receptor-like kinase from *Arabidopsis thaliana*. Therefore the novel gene was denominated a ROOT CLAVATA HOMOLOG 1 gene (RCH1).

An amino acid sequence alignment of the RCH1 protein and CLAVATA 1 from *A.*  
20 *thaliana* is shown in Figure 10. Calculation of the percentage of identical amino acids residues between RCH1 and CLV1 teaches that RCH1 is 39% identical to CLV1. Additional to 39% of identical amino acids, there is 49% of the amino acids that belong to the same class of amino acids and therefore have the same physical and chemical properties.

- 25 The classification of RCH1 as a novel type of CLAVATA is documented further in figures 11 and 12.

The conclusions we can draw from figure 11 is that based on the sequence, RCH1 is closer related to CLV1 compared to other known CLV1 homologues, such as ERECTA, BRASSINOLIDE INSENSITIVE and HAESA.

- 30 The fact that the novel LRR receptor like protein described in the present invention is rather a root clavata 1 homologue then another kind of LRR receptor-like kinase (e.g. resistance genes) is illustrated in Figure 12 in which the evolutionary relationship between clavata homologues and resistance genes is shown. The resistance genes



(RPS5, RPS2, RPP8, RPM1, RPS4, N, RPP5) are described in "Meyers BC, Dickerman AW, Michelmore RW, Sivaramakrishnan S, Sobral BW, Young ND. Plant disease resistance genes encode members of an ancient and diverse protein family within the nucleotide-binding super family. Plant J. 1999 Nov;20(3):317-32".

5 The present invention relates to a ROOT *CLAVATA* HOMOLOG 1 gene that differs from other known *CLAVATA*-like genes, in the fact that it is active in the root meristem, whereas the other known *CLAVATA* genes and *CLAVATA* homologs (like *ERECTA*, *BRASSINOLIDE* *INSENSITIVE* and *HAESA*) are only active in the shoot and floral meristem.

10 According to another embodiment, the invention also relates to nucleic acids encoding a functional plant LRR receptor-like kinase comprising one or more protein regions, distinguishing said plant LRR receptor-like kinase from those LRR receptor-like kinases known in the art.

The inventors found that the predicted amino acid sequence (BAB10317.1, receptor protein kinase-like protein) corresponding to the database entry with accession number  
15 AB017061 (*A. thaliana* chromosome 5), was missing a part of the amino acid sequence (namely, 25 amino acids as represented in SEQ ID NO. 5 of the present patent application). This extra stretch of 25 amino acids was identified by the cloning and the sequencing of the corresponding cDNA of the present invention. Therefore, the  
20 predicted prior art sequence is believed not to represent a functional enzyme.

The invention thus relates to an isolated nucleic acid selected from the group consisting of:

- (a) a nucleic acid consisting of at least part, preferably at least a functional part, of the DNA sequence as given in SEQ ID NO 1 or 3, or the complement thereof,
- 25 (b) a nucleic acid encoding a protein as given in SEQ ID NO 4 or 19 or encoding a fragment of said protein, wherein said fragment comprises the sequence as represented in SEQ ID NO 5, and
- (c) a nucleic acid encoding a protein with an amino acid sequence which is at least 60%, preferably at least 65 %, 70 %, 75%, 80%, 85%, more preferably at least  
30 90% or 95% identical to the protein as given in SEQ ID NO 4 or 9, wherein said amino acid sequence comprises the sequence as represented in SEQ ID NO 5,

characterised in that said nucleic acid encodes a novel LRR receptor-like kinase protein or an immunologically active and/or functional fragment of such a protein, and further provided that said nucleic acid is not one of the nucleic acids as deposited under the GenBank Accession numbers AB017061 or AQ966419.

5 The present invention also relates to an isolated LRR receptor-like kinase (protein) comprising one of the polypeptides selected from the group consisting of:

- (a) a polypeptide as given in SEQ ID NO 4 or SEQ ID NO 19,
- (b) a polypeptide comprising the amino acid sequence as given in SEQ ID NO 5, and,
- 10 (c) a polypeptide comprising the sequence represented in SEQ ID NO 5 and encoded by a nucleic acid as given in SEQ ID NO 1 or 3,

or a homologue or a derivative of said protein, or a fragment or an immunologically active and/or functional fragment thereof, provided that said homologue is not the amino acid sequence as described under the GenBank Accession number BAB10317.1.

15 With the expression "homologue" when referring to homologues of the LRR receptor-like kinase (protein), a polypeptide is meant which is at least 65% identical to the amino acid sequence of RCH1 as represented in SEQ ID NO 4 or 19.

More preferred, the invention relates to a protein consisting of an amino acid sequence as given in SEQ ID NO 4 or SEQ ID NO 19. More specifically, the present invention  
20 relates to a root CLAVATA homologue or a functional homologue thereof. A preferred fragment of said protein is represented in SEQ ID NO 5.

A further embodiment of the invention comprises homologues, derivatives, immunologically active and/or functional fragments of the LRR receptor-like kinase (protein) according to the invention, and proteins comprising said homologues,  
25 derivatives, immunologically active and/or functional fragments of said LRR receptor like kinase (protein).

As such, the present invention also relates to an isolated polypeptide comprising the sequence represented in SEQ ID NO 5 encodable by a nucleic acid molecule of the invention as defined above, or a homologue or a derivative thereof, or a fragment or an  
30 immunologically active and/or functional fragment thereof.

In a more preferred embodiment, the invention relates to a polypeptide, encodable by a nucleic acid molecule of the invention and which has an amino acid sequence as given in SEQ ID NO 4 or SEQ ID NO 19 or comprising an amino acid sequence as given in

SEQ ID NO 5, or a homologue or a derivative thereof, or an immunologically active and/or functional fragment thereof.

Any of said proteins can be produced in a biological system, e.g. a cell culture. Alternatively any of said proteins is chemically manufactured e.g. by solid phase peptide  
5 synthesis. Said proteins or fragments thereof can be part of a fusion protein as is the case in e.g. a two-hybrid assay which enables e.g. the identification of proteins interacting with the LRR receptor-like kinase according to the present invention.

Therefore, according to another embodiment, the invention also relates to a method for producing a polypeptide or a protein of the invention comprising culturing a host cell  
10 further specified above under conditions allowing the expression of the polypeptide and recovering the produced polypeptide from the culture.

The proteins or fragments thereof obtained by a method of the invention are furthermore useful e.g. to modulate the interaction between a LRR receptor-like kinase according to the invention and a ligand of the receptor and/or other identified interacting protein  
15 partners. Chemically synthesized peptides are particularly useful e.g. as a source of antigens for the production of antisera and/or antibodies.

The current invention thus furthermore encompasses antisera and/or antibodies specifically recognizing the LRR receptor-like kinase according to the invention or immunologically active parts or epitopes thereof. Said antisera and/or antibodies are  
20 useful in many areas related to the invention including: (i) identification in any organism, preferably plants, of other LRR receptor-like kinase and their genes according to the invention; (ii) quantification of synthesis in organisms and/or recombinant organisms of the LRR receptor-like kinase according to the invention; (iii) purification of the LRR receptor-like kinase according to the invention; (iv) immunoprecipitation of the LRR  
25 receptor-like kinase according to the invention e.g. as a way to identify other protein partners complexing with said LRR receptor-like kinase; (v) immunolocalization of the LRR receptor-like kinase according to the invention which is expressed in an organism or a recombinant organism.

The invention also relates to a method for the production of transgenic plants, plant cells  
30 or plant tissues comprising the introduction of a nucleic acid molecule of the invention in an expressible format or a vector as described earlier in said plant, plant cell or plant tissue

The invention also relates to a method as described above further comprising regenerating a plant from said plant cell.

In a further embodiment, the invention also relates to a transgenic plant cell comprising a nucleic acid sequence of the invention which is operably linked to regulatory elements  
5 allowing transcription and/or expression of said nucleic acid in plant cells or obtainable by one of the methods described above. Preferably, in said transgenic plant cell one of the nucleic acids of the invention is stably integrated into the genome of said plant cell. The invention further relates to a transgenic plant or plant tissue comprising said plant cells and to a harvestable part of said plant or tissue. Said harvestable part of said plant  
10 or tissue is preferably selected from the group consisting of seeds, leaves, fruits, stem cultures, rhizomes, tubers and bulbs.

The invention further extends to the progeny derived from any of the plants or plant parts described above.

The present invention is applicable to any plant, in particular a monocotyledonous plants  
15 and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia spp.*, *Acer spp.*, *Actinidia spp.*, *Aesculus spp.*, *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon spp.*, *Arachis spp.*, *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plurijuga*, *Betula spp.*, *Brassica spp.*, *Bruguiera gymnorrhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra spp.*, *Camellia sinensis*, *Canna indica*, *Capsicum spp.*, *Cassia spp.*,  
20 *Centroema pubescens*, *Chaenomeles spp.*, *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronillia varia*, *Cotoneaster serotina*, *Crataegus spp.*, *Cucumis spp.*, *Cupressus spp.*, *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*, *Cymbopogon spp.*, *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*,  
25 *Davallia divaricata*, *Desmodium spp.*, *Dicksonia squarosa*, *Diheteropogon amplexans*, *Dioclea spp.*, *Dolichos spp.*, *Dorycnium rectum*, *Echinochloa pyramidalis*, *Ehrartia spp.*, *Eleusine coracana*, *Eragrestis spp.*, *Erythrina spp.*, *Eucalyptus spp.*, *Euclea schimperi*, *Eulalia villosa*, *Fagopyrum spp.*, *Feijoa sellowiana*, *Fragaria spp.*, *Flemingia spp.*, *Freycinetia banksii*, *Geranium thunbergii*, *Ginkgo biloba*, *Glycine javanica*, *Gliricidia spp.*,  
30 *Gossypium hirsutum*, *Grevillea spp.*, *Guibourtia coleosperma*, *Hedysarum spp.*, *Hemarthia altissima*, *Heteropogon contortus*, *Hordeum vulgare*, *Hyparrhenia rufa*, *Hypericum erectum*, *Hyperthelia dissoluta*, *Indigo incarnata*, *Iris spp.*, *Leptarrhena pyrolifolia*, *Lespedeza spp.*, *Lettuca spp.*, *Leucaena leucocephala*, *Loudetia simplex*,

*Lotonous bainesii*, *Lotus* spp., *Macrotyloma axillare*, *Malus* spp., *Manihot esculenta*,  
*Medicago sativa*, *Metasequoia glyptostroboides*, *Musa sapientum*, *Nicotianum* spp.,  
*Onobrychis* spp., *Ornithopus* spp., *Oryza* spp., *Peltophorum africanum*, *Pennisetum*  
5 *cookianum*, *Photinia* spp., *Picea glauca*, *Pinus* spp., *Pisum sativum*, *Podocarpus totara*,  
*Pogonarthria fleckii*, *Pogonarthria squarrosa*, *Populus* spp., *Prosopis cineraria*,  
*Pseudotsuga menziesii*, *Pterolobium stellatum*, *Pyrus communis*, *Quercus* spp.,  
*Rhaphiolepis umbellata*, *Rhopalostylis sapida*, *Rhus natalensis*, *Ribes grossularia*,  
*Ribes* spp., *Robinia pseudoacacia*, *Rosa* spp., *Rubus* spp., *Salix* spp., *Schyzachyrium*  
10 *sanguineum*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Sequoiadendron*  
*giganteum*, *Sorghum bicolor*, *Spinacia* spp., *Sporobolus fimbriatus*, *Stiburus*  
*alopecuroides*, *Stylosanthos humilis*, *Tadehagi* spp., *Taxodium distichum*, *Themeda*  
*triandra*, *Trifolium* spp., *Triticum* spp., *Tsuga heterophylla*, *Vaccinium* spp., *Vicia*  
*spp.*, *Vitis vinifera*, *Watsonia pyramidata*, *Zantedeschia aethiopica*, *Zea mays*, amaranth,  
15 artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot, cauliflower,  
celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean,  
straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, amongst others, or  
the seeds of any plant specifically named above or a tissue, cell or organ culture of any  
of the above species.

20 Yet according to another embodiment the present invention relates to a method for  
conferring root-specificity and/or root-meristem-specificity and/or root-vascular-tissue-  
specificity and/or root-endodermis-specificity and/or root-cortex-specificity and/or root-  
epidermis-specificity and/or lateral-root cap-specificity and/or abundant expression in  
roots to other promoter sequences comprising the fusion of at least part of the DNA  
25 sequence as given in SEQ ID NO 1, 2 or 18 to a (second) transcriptional regulatory  
promoter sequence normally not exhibiting root-specificity.

The invention further relates to method for root-specific expression of a gene(s) of  
interest comprising operably linking of said gene(s) of interest to a transcriptional  
regulatory root-specific promoter comprising at least part of the nucleic acids as given in  
30 SEQ ID NO 1, 2 or 18 as defined above, and possibly in combination/or a (second)  
transcriptional regulatory promoter sequence not normally exhibiting root-specificity, as  
defined earlier.

The invention further relates to a method for modifying cell fate and/or plant development and/or plant morphology and/or biochemistry and/or physiology comprising the modification of expression in the meristem of the main and the lateral roots, or in the vascular tissue of the root, or in the lateral root primordia of a gene(s) of interest operably linked to a transcriptional regulatory root promoter as defined earlier.

The ability to drive tissue-specific expression of any gene of interest in plants is considered to be of great agronomic importance. This art can be used to exclude expression in eatable parts of the plant, which averts the uptake of ectopic components. Alternatively the expression of the transgene can be restricted to the relevant tissues of the plant so the total organism does not spend too much energy in superfluous production of the foreign component. This technique can also reduce potential yield loss by limiting the expression of some pernicious, yet useful agronomic genes, to specific tissues only. Said genes of interest, which can be expressed in a root-specific manner under the control of the present invention can also be involved in phytoremediation, enhanced root-growth (like cell cycle genes or cytokinin oxidase) or disease tolerance. These methods are more elaborated in detail below.

The invention described here provides a new transcriptional regulatory element which can be operably linked to a gene effecting and/or modifying root metabolites which can be involved in phytoremediation. Examples are genes involved in the production of citric and maleic acids that can be secreted by the plant and subsequently form complexes with the aluminium in the soil. Root specific expression of the aluminium-resistance gene that give rise to said metabolites can therefore allow plants to grow on acid soils, like those of south-eastern United States, Central and south America, North Africa and parts of India and China, where the aluminium is set free as an ion that poisons plant roots.

Alternatively, the promoter of the present invention also provides a tool to be used in a strategy for environmental remediation. Accordingly, a metal-resistance gene such as the TaPCS1 or the CAD1 gene, contributing cadmium tolerance, can be expressed specifically in roots to enable enhanced growth of transgenic plants on soils contaminated with cadmium. The metals can consequently be extracted from the soil and stored in the plant material, which could then be harvested and incinerated.

Therefore according to preferred embodiments, the present invention relates to a method for phytoremediation or environmental remediation comprising the expression of

a gene(s) of interest under the control of a transcriptional regulatory root promoter as defined earlier.

In conclusion the present invention provides a method for broadening the range of soils or environments in which a plant may thrive or that can be used for agriculture.

5 Plant root development is an essential determinant of plant growth and crop yield, so that yield could be enhanced by induced changes in expression of root-specific regulatory factors. Cyclin D for example, which promotes the cell division, can be expressed under the root-specific control of the current invention and hence contribute to increased growth rate and crop yield. Also genes that are involved in biosynthesis and  
10 perception of phytohormones like auxins, cytokinines, ethylene, abscisic acid and gibberellins can be used to enhance root growth and development. However, the root-specific expression of these genes might be pivotal for the overall welfare of the transgenic plant.

It is known that the constitutive and ubiquitous expression of the cytokinin oxidase in  
15 *Arabidopsis thaliana* leads to the promotion of an enlarged root system and enhanced root biomass, but also to diminished shoot growth. To overcome this problem the cytokinin oxidase can be operably linked to the promoter of the present invention as described in example 6, which will enhance root growth without negatively affecting shoot growth. This enhanced root growth in the plant can be used for bioremediation or  
20 phytoremediation.

Also the said gene of interest, that can be operably linked to the promoter of the present invention, can be a root transcription factor, such as Alfin. Alfin binds to promoter fragments of predominantly root-specific and salt inducible genes and its activity has major implications for crop plant yield (Winicov 2000) (Winicov & Bastola 1999)). In  
25 conclusion the present invention provides a method for enhancing plant growth and crop yield.

It is also clear for the skilled artisan that the promoter of the present invention provides a useful tool to be used for the introduction of resistance to soil-borne pathogen attack to plants, a widespread target for genetic improvement of crop plants. Certain disease-  
30 inducing microorganisms attack the belowground plant tissue and any genetic modification to contribute resistance to such organisms will require expression of the resistance-gene in the roots. To overcome this problem, the coding region that is operably linked to the promoter of the present invention preferably encodes a protein

which is toxic to root-attacking organisms and more preferably the protein is an insecticidal endotoxin of *Bacillus thuringiensis*. Similarly to the above-mentioned pathogens, there are pests attacking plants. Certain insect species attack green leaf tissue, whereas other, for example coleoptera, attack the roots. Corn root worm  
5 (*Diabrotica undecimpunctata howardi* Barber) for example is a particularly difficult pest to control or to eradicate. It attacks the plant below the soil line, where insecticides are difficult or impossible to apply effectively. Corn root worm can be eradicated by root-specific expression of the Cry III gene, which produces a component, specifically toxic for coleoptera. Also root-specific expression of the limonene cyclase gene in  
10 combination with GPPP synthetase would be larvicidal. Another pest, nematodes, that penetrate the root and form root-knots or cysts, which cause severe damage to crops throughout the world, could be treated with the root-specific expression of nematode resistance genes such as Hs1<sup>pro-1</sup>, Ni-1 and Gpa2.

In conclusion the invention disclosed here provides a method for conferring enhanced  
15 resistance to pathogens to a transgenic plant which pathogens attack the belowground plant tissue comprising the expression of a gene(s) of interest under the control of a transcriptional regulatory root-specific promoter as defined earlier.

In yet another embodiment of the invention, the invention provides a method to enhanced freezing tolerance comprising the expression of a gene(s) of interest under  
20 the control of a transcriptional regulatory root-specific promoter as defined earlier. Preferred is the overexpressing of the *Arabidopsis thaliana* DNA binding factors CBF1 and DREB1A that induce cold-regulated (COR) genes (Jaglo-Ottosen et al. 1998). However, constitutive expression of these genes in all organs of the plant led to growth retardation under normal growth conditions. Root-specific expression of these freezing-  
25 tolerance genes under control of the promoter sequence of the present invention can make the plant tolerant to cold temperatures of the soil without affecting the growth of the plant. This technique can have important agronomic potential since lots of soils are inaccessible for crop production because of presence of permanently frozen layers in the soil.

30 Alternatively, other genes of interest can tolerize the transgenic plant for other weather conditions like drought (e.g. oat arginin decarboxylase gene) and high salt conditions (e.g. MtID gene). In conclusion, the present invention provides a method to contribute to a transgenic plant stress-tolerance.



Further advantages to root-preferred gene expression include the production of useful proteins in an industrial setting. Light-sensitive proteins may be synthesized in root tissue such that said proteins are not exposed to light. Therefore the present invention also relates to a method for the production of light-sensitive proteins comprising the  
5 expression of a gene encoding said light-sensitive protein under the control of a transcriptional regulatory root-specific promoter of the invention.

Also considered as an embodiment of the invention is the use of the RCH1 promoter for applications based on the inhibition of expression of native DNA sequences within the plant's root to achieve a desired phenotype resulting from the silencing of the native  
10 gene. In this case, such inhibition might be accomplished with transformation of the plant to comprise a transcriptional regulatory root-specific promoter of the present invention operably linked to e.g. an antisense nucleotide sequence to the gene of interest, a gene silencing construct or a ribozyme.

Those skilled in the art will be aware that plants secure the formation of organs  
15 throughout their life span by developing and maintaining a collection of stem cells termed the meristem. The promoter of the present invention offers the opportunity to deliver a certain protein specifically to these cells. For example overexpression of genes that influence the proliferation and/or the differentiation of the root meristem could contribute to a higher turnover of the meristem cells and therefor enhance the root  
20 architecture.

The promoter of the present invention drives the *Arabidopsis thaliana* gene ROOT CLAVATA HOMOLOG 1 (RCH1). Since the *clavata*-genes are key role players in shoot-meristem maintenance, persons skilled in the art may recognize the possible involvement of the RCH1 operon in root meristem maintenance. Therefor one could  
25 assume that the control of this promoter is influenced by signals that promote meristem maintenance. This might be an advantage when aiming to stimulate the root meristem-formation, which eventually could result in more roots, higher uptake of resources and in higher crop yield.

Therefore, according to preferred embodiments, the invention relates to methods for  
30 stimulating meristem formation and/or maintaining root meristem comprising the expression of a gene that influences the proliferation and/or differentiation of the root meristeme under the control of a transcriptional regulatory root (-specific) promoter of the invention.

The present invention therefore relates to a method for stimulating root meristem formation or for root-meristeme maintenance comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase, for instance the LRR receptor-like kinase of the invention, wherein optionally said  
5 nucleic acid is operably linked to a plant-operable promoter sequence, for instance said plant-operable promoter being the transcriptional regulatory root promoter as defined earlier.

As mentioned supra, the combined gene expression data and homology to CLAVATA 1 indicate that the novel plant LRR receptor-like kinase of the invention, RCH1 (SEQ ID  
10 No. 3, 4, 19), is very likely to exert a function that is involved in the development and/or maintenance of the root meristem. As the root is the main plant organ for nutrient uptake from the environment, enhanced root formation could eventually lead to overall growth enhancement and better yield. Therefore preferred genes to be expressed in the methods for stimulating meristem formation and/or for root meristeme maintenance are  
15 genes encoding the polypeptide(s) of the invention.

The present invention also relates to a method for enhancing root formation and/or root growth comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase, for instance the LRR receptor-like  
20 kinase of the invention, wherein optionally said nucleic acid is operably linked to a plant-operable promoter sequence, for instance said plant-operable promoter being the transcriptional regulatory root promoter as defined earlier.

The present invention therefore relates to a method for enhancing overall growth and yield comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase, for instance the LRR receptor-like  
25 kinase of the invention, wherein optionally said nucleic acid is operably linked to a plant-operable promoter sequence, for instance said plant-operable promoter being the transcriptional regulatory root promoter as defined earlier.

The present invention also relates to a method for enhancing overall growth and yield comprising the expression of a protein of the invention under the control of a  
30 transcriptional regulatory root (or root-specific) promoter of the invention.

Also in yet other embodiments of the invention, methods are provided for modifying cell fate and/or plant development and/or plant morphology and/or biochemistry and/or

physiology comprising the modification of expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase, for instance the LRR receptor-like kinase of the invention and defined above, wherein optionally said nucleic acid is operably linked to a plant-operable promoter sequence, for instance said plant-operable promoter being the transcriptional regulatory root promoter as defined earlier.

The present invention further relates to a method to confer pathogen resistance to a transgenic plant comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase, for instance the LRR receptor-like kinase of the invention and defined above, wherein optionally said nucleic acid is operably linked to a plant-operable promoter sequence, for instance said plant-operable promoter being the transcriptional regulatory root promoter as defined earlier.

Many pathogen resistance genes are LRR receptor-like proteins, which initiate a signal transduction pathway in the root cells, leading to the activation of defense mechanisms against the pathogen. The novel LRR receptor-like kinase might contribute to pathogen resistance to the transgenic plant when transformed herein.

Yet another embodiment of the invention relates to methods to perform a functional analysis of the DNA sequence according to the present invention, encoding a novel LRR receptor-like protein. For persons skilled in the art it is possible to make single or double knock-out mutants of *Arabidopsis thaliana*, wherein the DNA of the present invention is inactivated. Also introduction of the antisense sequence of the DNA of the invention, as well as the overexpression of its sense sequence in a transgenic plant can elucidate a specific feature in this transgenic plant. Furthermore, the loss of function of this gene of the invention by introduction of dominant negative mutations can result in interesting characteristics of the mutant plant.

Another aspect of the present invention is the revelation of a new target for herbicides or growth regulators. When said gene of the invention is an essential gene for plant cell viability, it can be a target for a herbicide or growth regulator.

Therefore the invention relates to a method for identifying and obtaining proteins interacting with a (LRR receptor-like kinase) polypeptide of the invention comprising a screening assay wherein said polypeptide is used or expressed.

In a preferred embodiment the invention relates to a method for identifying and obtaining proteins interacting with a an LRR receptor-like kinase (protein) comprising a two-hybrid

screening system wherein a nucleic acid encoding a polypeptide of the invention as a bait and a cDNA library as prey are expressed.

The invention also relates to a method for modulating the interaction between an LRR receptor-like kinase (protein) of the invention and interacting proteins obtainable by a method as described above.

The invention further relates to a method for identifying and obtaining compounds interacting with an LRR receptor-like kinase (protein) comprising the steps of:

- a) providing a two-hybrid screening system wherein a polypeptide or protein of the invention and a protein interacting with said (LRR receptor-like kinase) polypeptide or protein or an interacting protein obtainable by a method of claim as claimed above are expressed,
- b) interacting said compound with the complex formed by the expressed proteins as defined in a),
- c) detecting a second complex, wherein the presence of said second complex identifies a compound which specifically binds to one of said polypeptides or said second complex, and
- d) identifying the compound.

The invention also relates to a method for identifying compounds or mixtures of compounds which specifically bind to a polypeptide of the invention, comprising:

- a) combining a polypeptide of the invention with said compound or mixtures of compounds under conditions suitable to allow complex formation, and,
- b) detecting complex formation, wherein the presence of a complex identifies a molecule which specifically binds said polypeptide.

The invention further relates to the use of a molecule identified by means of one of the methods described above as a plant growth regulator or herbicide.

The invention also relates to a method for the production of a plant growth regulator or herbicide composition comprising the steps of one of the methods described above and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture.

The invention also relates to the use of any of the nucleic acid molecules, vectors, polypeptides or antibodies of described herein for modifying cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology.

The invention also relates to a diagnostic composition comprising at least one of the nucleic acid molecules, vectors, polypeptides or antibodies of the invention.

It is further important to notice that CLAVATA 1 has many analogues in other plant species, so it is likely that also the promoter of RCH1, the RCH1 ORF or the complete  
5 RCH1 operon can be functional in those other plant species, and possibly exert the same specific activation pattern. This can be investigated by root-specific reporter gene expression in rice mediated by the *Arabidopsis thaliana* RCH1 promoter as described in example 5.

### Definitions and elaborations to the embodiments

10 The terms "protein(s)", "peptide(s)" or "oligopeptide(s)", when used herein refer to amino acids in a polymeric form of any length. Said terms also include known amino acid modifications such as disulphide bond formation, cysteinylation, oxidation, glutathionylation, methylation, acetylation, farnesylation, biotinylation, stearylation, formylation, lipoic acid addition, phosphorylation, sulphation, ubiquitination,  
15 myristoylation, palmitoylation, geranylgeranylation, cyclization (e.g. pyroglutamic acid formation), oxidation, deamidation, dehydration, glycosylation (e.g. pentoses, hexosamines, N-acetylhexosamines, deoxyhexoses, hexoses, sialic acid etc.), acylation and radiolabels (e.g. <sup>125</sup>I, <sup>131</sup>I, <sup>35</sup>S, <sup>14</sup>C, <sup>32</sup>P, <sup>33</sup>P, <sup>3</sup>H) as well as non-naturally occurring amino acid residues, L-amino acid residues and D-amino acid residues.

20 "Homologues" or "Homologs" of a protein of the invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which contain amino acid substitutions, deletions and/or additions relative to the said protein with respect to which they are a homolog, without altering one or more of its functional properties, in particular without reducing the activity of the resulting. For example, a homolog of said protein will  
25 consist of a bioactive amino acid sequence variant of said protein. To produce such homologs, amino acids present in the said protein can be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic moment, antigenicity, propensity to form or break  $\alpha$ -helical structures or  $\beta$ -sheet structures, and so on. An overview of physical and chemical properties of amino acids is  
30 given in Table 1.

Substitutional variants of a protein of the invention are those in which at least one residue in said protein amino acid sequence has been removed and a different residue

inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about 1-20 residues. Preferably, amino acid substitutions will comprise conservative amino acid substitutions, such as those described *supra*.

**Table 1: Properties of naturally occurring amino acids.**

Charge properties / hydrophobicity	Side group	Amino Acid
nonpolar hydrophobic	Aliphatic Aliphatic, S-containing Aromatic Imino	Ala, Ile, Leu, Val Met Phe, Trp Pro
polar uncharged	Aliphatic Amide Aromatic Hydroxyl Sulfhydryl	Gly Asn, Gln Tyr Ser, Thr Cys
positively charged	Basic	Arg, His, Lys
negatively charged	Acidic	Asp, Glu

Insertional amino acid sequence variants of a protein of the invention are those in which one or more amino acid residues are introduced into a predetermined site in said protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino or carboxyl terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)<sub>6</sub>-tag, glutathione S-transferase, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100

epitope (EETARFQPGYRS), c-myc epitope (EQKLISEEDL), FLAG<sup>®</sup>-epitope (DYKDDDK), lacZ, CMP (calmodulin-binding peptide), HA epitope (YPYDVPDYA), protein C epitope (EDQVDPRLIDGK) and VSV epitope (YTDIEMNRLGK).

Deletional variants of a protein of the invention are characterised by the removal of one or more amino acids from the amino acid sequence of said protein.

Homologues of the peptides or polypeptides of the invention contain a number of amino acid substitutions, deletions and/or additions relative to said peptide or polypeptide such that said homologues are still substantially different from the peptides or polypeptides known in the art. The difference between proteins can be calculated in terms of % identity according to methods well known in the art. Preferably, the homologues of the peptides or polypeptides of the present invention comprise the amino acid sequence represented in SEQ ID NO 5, optionally comprising minor amino acid substitutions such as those described in table 1. Other homologues of the polypeptides of the invention have an amino acid sequence which is at least 65 % identical to the sequence represented in SEQ ID NO 4 or 19.

Amino acid variants of a protein of the invention may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by recombinant DNA manipulations. The manipulation of DNA sequences to produce variant proteins which manifest as substitutional, insertional or deletional variants are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA having known sequence are well known to those skilled in the art, such as by M13 mutagenesis, T7-Gen in vitro mutagenesis kit (USB, Cleveland, OH), QuickChange Site Directed mutagenesis kit (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

"Derivatives" of a protein of the invention are those peptides, oligopeptides, polypeptides, proteins and enzymes which comprise at least about five contiguous amino acid residues of said polypeptide but which retain the biological activity of said protein and which may further comprise additional naturally-occurring, altered glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of said polypeptide. Alternatively or in addition, a derivative may comprise one or more non-amino acid substituents compared to the amino acid sequence of a naturally-occurring form of said polypeptide, for

example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound thereto to facilitate its detection. Preferably, the derivatives of the proteins of the present invention comprise the amino acid sequence as represented in SEQ ID NO 5.

5 With "immunologically active" is meant that a molecule or specific fragments thereof such as epitopes or haptens are recognized by, i.e. bind to antibodies. Preferably, the immunologically active or fragments of the proteins of the present invention comprise the amino acid sequence as represented in SEQ ID NO 5.

With "functional fragment" or "functional homologue" is meant a protein which comprises  
10 at least about 5, 10, 20, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1050, 1100, 1110, 1120, 1130, 1131, 1132, 1133 or 1134 contiguous amino acid residues of said polypeptide but which retains the biological activity of said protein. Preferably, the functional fragments or functional homologues of the polypeptide or proteins of the invention comprise the amino acid sequence as represented in SEQ ID NO 5 but  
15 wherein minor amino acid substitutions are allowed (see table 1).

In the context of the current invention are embodied homologs, derivatives and/or immunologically active fragments of any of the inventive LRR receptor-like kinase protein or polypeptide or homolog, derivative or fragment thereof as defined supra.

"Antibodies" include monoclonal, polyclonal, synthetic or heavy chain camel antibodies  
20 as well as fragments of antibodies such as Fab, Fv or scFv fragments. Monoclonal antibodies can be prepared by the techniques as described in e.g. Liddle and Cryer (1991) which comprise the fusion of mouse myeloma cells to spleen cells derived from immunized animals. Furthermore, antibodies or fragments thereof to a molecule or fragments thereof can be obtained by using methods as described in e.g. Harlow and  
25 Lane (1988). In the case of antibodies directed against small peptides such as fragments of a protein of the invention, said peptides are generally coupled to a carrier protein before immunization of animals. Such protein carriers include keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin and Tetanus toxoid. The carrier protein enhances the immune response of the animal and provides epitopes for  
30 T-cell receptor binding sites. The term "antibodies" furthermore includes derivatives thereof such as labelled antibodies. Antibody labels include alkaline phosphatase, PKH2, PKH26, PKH67, fluorescein (FITC), Hoechst 33258, R-phycoerythrin (PE), rhodamine (TRITC), Quantum Red, Texas Red, Cy3, biotin, agarose, peroxidase, gold



spheres and radiolabels (e.g.  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$ ,  $^{14}\text{C}$ ,  $^{32}\text{P}$ ,  $^{33}\text{P}$ ,  $^3\text{H}$ ). Tools in molecular biology relying on antibodies against a protein include protein gel blot analysis, screening of expression libraries allowing gene identification, protein quantitative methods including ELISA and RIA, immunoaffinity purification of proteins, immunoprecipitation of proteins  
5 (e.g. Magyar et al. 1997) and immunolocalization. Other uses of antibodies and especially of peptide antibodies include the study of proteolytic processing (Löffler et al. 1994, Woulfe et al. 1994), determination of protein active sites (Lerner 1982), the study of precursor and post-translational processing (Baron and Baltimore 1982, Lerner et al. 1981, Semler et al. 1982), identification of protein domains involved in protein-protein  
10 interactions (Murakami et al. 1992) and the study of exon usage in gene expression (Tamura et al. 1991).

Embodied in the current invention are antibodies recognizing a LRR receptor-like kinase polypeptide or protein, or homolog, derivative or fragment thereof as defined supra or specific epitopes of said polypeptide or protein.

15 The terms "gene(s)", "polynucleotide(s)", "nucleic acid sequence(s)", "nucleotide sequence(s)", "DNA sequence(s)" or "nucleic acid molecule(s)", when used herein refer to nucleotides, either ribonucleotides or deoxyribonucleotides or a combination of both, in a polymeric form of any length. Said terms furthermore include double-stranded and single-stranded DNA and RNA. Said terms also include known nucleotide modifications  
20 such as methylation, cyclization and 'caps' and substitution of one or more of the naturally occurring nucleotides with an analog such as inosine. Modifications of nucleotides include the addition of acridine, amine, biotin, cascade blue, cholesterol, Cy3<sup>®</sup>, Cy5<sup>®</sup>, Cy5.5<sup>®</sup> Dabcyl, digoxigenin, dinitrophenyl, Edans, 6-FAM, fluorescein, 3'-glyceryl, HEX, IRD-700, IRD-800, JOE, phosphate psoralen, rhodamine, ROX, thiol  
25 (SH), spacers, TAMRA, TET, AMCA-S<sup>®</sup>, SE, BODIPY<sup>®</sup>, Marina Blue<sup>®</sup>, Pacific Blue<sup>®</sup>, Oregon Green<sup>®</sup>, Rhodamine Green<sup>®</sup>, Rhodamine Red<sup>®</sup>, Rhodol Green<sup>®</sup> and Texas Red<sup>®</sup>. Polynucleotide backbone modifications include methylphosphonate, 2'-OMe-methylphosphonate RNA, phosphorothiorate, RNA, 2'-OMeRNA. Base modifications include 2-amino-dA, 2-aminopurine, 3'-(ddA), 3'dA(cordycepin), 7-deaza-dA, 8-Br-dA, 8-oxo-dA, N<sup>6</sup>-Me-dA, abasic site (dSpacer), biotin dT, 2'-OMe-5Me-C, 2'-OMe-propynyl-C,  
30 3'-(5-Me-dC), 3'-(ddC), 5-Br-dC, 5-I-dC, 5-Me-dC, 5-F-dC, carboxy-dT, convertible dA, convertible dC, convertible dG, convertible dT, convertible dU, 7-deaza-dG, 8-Br-dG, 8-oxo-dG, O<sup>6</sup>-Me-dG, S6-DNP-dG, 4-methyl-indole, 5-nitroindole, 2'-OMe-inosine, 2'-dl, O<sup>6</sup>-

phenyl-dl, 4-methyl-indole, 2'-deoxynebularine, 5-nitroindole, 2-aminopurine, dP(purine analogue), dK(pyrimidine analogue), 3-nitropyrrole, 2-thio-dT, 4-thio-dT, biotin-dT, carboxy-dT, O<sup>4</sup>-Me-dT, O<sup>4</sup>-triazol dT, 2'-OMe-propynyl-U, 5-Br-dU, 2'-dU, 5-F-dU, 5-I-dU, O<sup>4</sup>-triazol dU and radiolabels (e.g. <sup>125</sup>I, <sup>131</sup>I, <sup>35</sup>S, <sup>14</sup>C, <sup>32</sup>P, <sup>33</sup>P, <sup>3</sup>H). Said terms also encompass peptide nucleic acids (PNAs), a DNA analogue in which the backbone is a pseudopeptide consisting of N-(2-aminoethyl)-glycine units rather than a sugar. PNAs mimic the behaviour of DNA and bind complementary nucleic acid strands. The neutral backbone of PNA results in stronger binding and greater specificity than normally achieved. In addition, the unique chemical, physical and biological properties of PNA have been exploited to produce powerful biomolecular tools, antisense and antigene agents, molecular probes and biosensors. With "recombinant DNA molecule" or "chimeric gene" is meant a hybrid DNA produced by joining pieces of DNA from different sources. With "heterologous nucleotide sequence" is intended a sequence that is not naturally occurring with the promoter sequence. While this nucleotide sequence is heterologous to the promoter sequence, it may be homologous, or native, or heterologous, or foreign, to the plant host. "Sense strand" refers to the strand of a double-stranded DNA molecule that is homologous to a mRNA transcript thereof. The "anti-sense strand" contains an inverted sequence which is complementary to that of the "sense strand".

A "coding sequence" or "open reading frame" or "ORF" is defined as a nucleotide sequence that can be transcribed into mRNA and/or translated into a polypeptide when placed under the control of appropriate regulatory sequences, i.e. when said coding sequence or ORF is present in an expressible format. Said coding sequence or ORF is bounded by a 5' translation start codon and a 3' translation stop codon. A coding sequence or ORF can include, but is not limited to RNA, mRNA, cDNA, recombinant nucleotide sequences, synthetically manufactured nucleotide sequences or genomic DNA. Said coding sequence or ORF can be interrupted by intervening nucleic acid sequences.

**Table 2. Degeneracy of the genetic code.**

Amino Acid	Three-letter code	One-letter code	Possible codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Asparagine	Asn	N	AAC	AAU					
Aspartic Acid	Asp	D	GAC	GAU					
Cysteine	Cys	C	UGC	UGU					
Glutamic Acid	Glu	E	GAA	GAG					
Glutamine	Gln	Q	CAA	CAG					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Lysine	Lys	K	AAA	AAG					
Methionine	Met	M	AUG						
Phenylalanine	Phe	F	UUC	UUU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					
Valine	Val	V	GUA	GUC	GUG	GUU			
			Possible "STOP" codons						
			UAA	UAG	UGA				

Genes and coding sequences essentially encoding the same protein but isolated from  
5 different sources can consist of substantially divergent nucleic acid sequences. Reciprocally, substantially divergent nucleic acid sequences can be designed to effect expression of essentially the same protein. Said nucleic acid sequences are the result of e.g. the existence of different alleles of a given gene, of the degeneracy of the genetic code or of differences in codon usage. Thus, as indicated in Table 2, amino acids such

as methionine and tryptophan are encoded by a single codon whereas other amino acids such as arginine, leucine and serine can each be translated from up to six different codons. Differences in preferred codon usage are illustrated below for *Agrobacterium tumefaciens* (a bacterium), *A. thaliana*, *M. sativa* (two dicotyledonous plants) and *Oryza sativa* (a monocotyledonous plant). These examples were extracted from (<http://www.kazusa.or.jp/codon>). To give one example, the codon GGC (for glycine) is the most frequently used codon in *A. tumefaciens* (36.2 ‰), is the second most frequently used codon in *O. sativa* but is used at much lower frequencies in *A. thaliana* and *M. sativa* (9 ‰ and 8.4 ‰, respectively). Of the four possible codons encoding glycine (see Table 6), said GGC codon is most preferably used in *A. tumefaciens* and *O. sativa*. However, in *A. thaliana* this is the GGA (and GGU) codon whereas in *M. sativa* this is the GGU (and GGA) codon.

"Hybridization" is the process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridization process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridization, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination. The hybridization process can also occur with one of the complementary nucleic acids immobilized to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridization process can furthermore occur with one of the complementary nucleic acids immobilized to a solid support such as a nitrocellulose or nylon membrane or immobilized by e.g. photolithography to e.g. a silicious glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridization, plaque hybridization, in situ hybridization and microarray hybridization. In order to allow hybridization to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridization is influenced by conditions such as temperature, salt concentration and hybridization buffer composition. High stringency conditions for hybridization include high

temperature and/or low salt concentration (salts include NaCl and Na<sub>3</sub>-citrate) and/or the inclusion of formamide in the hybridization buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridization buffer and/or exclusion of compounds such as dextran sulfate or polyethylene glycol (promoting molecular crowding) from the hybridization buffer. Conventional hybridization conditions are described in e.g. Sambrook et al. (1989) but the skilled craftsman will appreciate that numerous different hybridization conditions can be designed in function of the known or the expected homology and/or length of the nucleic acid sequence. Sufficiently low stringency hybridization conditions are particularly preferred to isolate nucleic acids heterologous to the DNA sequences of the invention defined supra. Elements contributing to said heterology include allelism, degeneration of the genetic code and differences in preferred codon usage as discussed supra.

Clearly, the current invention embodies the use of the inventive DNA sequences encoding a LRR receptor-like kinase, homolog, derivative and/or immunologically fragment thereof as defined higher in any method of hybridization. The current invention furthermore also relates to DNA sequences specifically hybridizing to said inventive DNA sequences.

DNA sequences as defined in the current invention can be interrupted by intervening sequences. With "intervening sequences" is meant any nucleic acid sequence which disrupts a coding sequence comprising said inventive DNA sequence or which disrupts the expressible format of a DNA sequence comprising said inventive DNA sequence. Removal of the intervening sequence restores said coding sequence or said expressible format. Examples of intervening sequences include introns, mobilizable DNA sequences such as transposons and DNA tags such as e.g. a T-DNA. With "mobilizable DNA sequence" is meant any DNA sequence that can be mobilized as the result of a recombination event.

To effect expression of a protein in a cell, tissue or organ, preferably of plant origin, either the protein may be introduced directly to said cell, such as by microinjection or ballistic means or alternatively, an isolated nucleic acid molecule encoding said protein may be introduced into said cell, tissue or organ in an expressible format.

Preferably, the DNA sequence of the invention comprises a coding sequence or open reading frame (ORF) encoding a LRR receptor-like kinase or a homolog or derivative thereof or an immunologically active fragment thereof as defined supra. The preferred

protein of the invention comprises the amino acid sequence of said LRR receptor-like kinase.

With "vector" or "vector sequence" is meant a DNA sequence which can be introduced in an organism by transformation and can be stably maintained in said organism. Vector maintenance is possible in e.g. cultures of *Escherichia coli*, *A. tumefaciens*, *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe*. Other vectors such as phagemids and cosmid vectors can be maintained and multiplied in bacteria and/or viruses. Vector sequences generally comprise a set of unique sites recognized by restriction enzymes, the multiple cloning site (MCS), wherein one or more non-vector sequence(s) can be inserted.

With "non-vector sequence" is accordingly meant a DNA sequence which is integrated in one or more of the sites of the MCS comprised within a vector.

"Expression vectors" form a subset of vectors which, by virtue of comprising the appropriate regulatory sequences enabling the creation of an expressible format for the inserted non-vector sequence(s), thus allowing expression of the protein encoded by said non-vector sequence(s). Expression vectors are known in the art enabling protein expression in organisms including bacteria (e.g. *E. coli*), fungi (e.g. *S. cerevisiae*, *S. pombe*, *Pichia pastoris*), insect cells (e.g. baculoviral expression vectors), animal cells (e.g. COS or CHO cells) and plant cells (e.g. potato virus X-based expression vectors, see e.g. Vance et al. 1998 - WO9844097).

The current invention clearly includes any vector or expression vector comprising a non-vector DNA sequence comprising the promoter sequence according to the present invention or a non)vector sequence encoding a LRR receptor-like kinase, homolog, derivative and/or immunologically active fragment thereof as defined supra.

As an alternative to expression vector-mediated protein production in biological systems, chemical protein synthesis can be applied. Synthetic peptides can be manufactured in solution phase or in solid phase. Solid phase peptide synthesis (Merrifield 1963) is, however, the most common way and involves the sequential addition of amino acids to create a linear peptide chain. Solid phase peptide synthesis includes cycles consisting of three steps: (i) immobilization of the carboxy-terminal amino acid of the growing peptide chain to a solid support or resin; (ii) chain assembly, a process consisting of activation, coupling and deprotection of the amino acid to be added to the growing peptide chain; and (iii) cleavage involving removal of the completed peptide chain from the resin and

removal of the protecting groups from the amino acid side chains. Common approaches in solid phase peptide synthesis include Fmoc/tBu (9-fluorenylmethyloxycarbonyl/t-butyl) and Boc (t-butyloxycarbonyl) as the amino-terminal protecting groups of amino acids. Amino acid side chain protecting groups include methyl (Me), formyl (CHO), ethyl (Et), acetyl (Ac), t-butyl (t-Bu), anisyl, benzyl (Bzl), trifluoroacetyl (Tfa), N-hydroxysuccinimide (ONSu, OSu), benzoyl (Bz), 4-methylbenzyl (Meb), thioanisyl, thiocresyl, benzyloxymethyl (Bom), 4-nitrophenyl (ONp), benzyloxycarbonyl (Z), 2-nitrobenzoyl (NBz), 2-nitrophenylsulphenyl (Nps), 4-toluenesulphonyl (Tosyl, Tos), pentafluorophenyl (Pfp), diphenylmethyl (Dpm), 2-chlorobenzyloxycarbonyl (Cl-Z), 2,4,5-trichlorophenyl, 2-bromobenzyloxycarbonyl (Br-Z), triphenylmethyl (Trityl, Trt), and 2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc). During chain assembly, Fmoc or Boc are removed resulting in an activated amino-terminus of the amino acid residue bound to the growing chain. The carboxy-terminus of the incoming amino acid is activated by conversion into a highly reactive ester, e.g. by HBTU. With current technologies (e.g. PerSeptive Biosystems 9050 synthesizer, Applied Biosystems Model 431A Peptide Synthesizer), linear peptides of up to 50 residues can be manufactured. A number of guidelines is available to produce peptides that are suitable for use in biological systems including (i) limiting the use of difficult amino acids such as cys, met, trp (easily oxidized and/or degraded during peptide synthesis) or arg; (ii) minimize hydrophobic amino acids (can impair peptide solubility); and (iii) prevent an amino-terminal glutamic acid (can cyclize to pyroglutamate).

By "expressible format" is meant that the isolated nucleic acid molecule is in a form suitable for being transcribed into mRNA and/or translated to produce a protein, either constitutively or following induction by an intracellular or extracellular signal, such as an environmental stimulus or stress (mitogens, anoxia, hypoxia, temperature, salt, light, dehydration, etc) or a chemical compound such as IPTG (isopropyl- $\beta$ -D-thiogalactopyranoside) or such as an antibiotic (tetracycline, ampicillin, rifampicin, kanamycin), hormone (e.g. gibberellin, auxin, cytokinin, glucocorticoid, brassinosteroid, ethylene, abscisic acid etc), hormone analogue (iodoacetic acid (IAA), 2,4-D, etc), metal (zinc, copper, iron, etc), or dexamethasone, amongst others. As will be known to those skilled in the art, expression of a functional protein may also require one or more post-translational modifications, such as glycosylation, phosphorylation, dephosphorylation,

or one or more protein-protein interactions, amongst others. All such processes are included within the scope of the term "expressible format".

Preferably, expression of a protein in a specific cell, tissue, or organ, preferably of plant origin, is effected by introducing and expressing an isolated nucleic acid molecule encoding said protein, such as a cDNA molecule, genomic gene, synthetic  
5 oligonucleotide molecule, mRNA molecule or open reading frame, to said cell, tissue or organ, wherein said nucleic acid molecule is placed operably in connection with suitable regulatory sequences including a promoter, preferably a plant-expressible promoter, and a terminator sequence.

10 "Regulatory sequence" refers to control DNA sequences which are necessary to affect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes generally control sequences include promoters, terminators and enhancers or silencers.  
15 The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components and which determines when, how much and where a specific gene is expressed.

Reference herein to a "promoter" is to be taken in its broadest context and includes the  
20 transcriptional regulatory sequences derived from a classical eukaryotic genomic gene, including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner.

25 The term "promoter" also includes the transcriptional regulatory sequences of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or a -10 box transcriptional regulatory sequences.

The term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell,  
30 tissue or organ.

The term "functional part" when referred to nucleic acids in relation to the above defined transcriptional regulatory sequences, refers to a part or parts of the nucleic acid having



the activity to specifically drive or promote transcription from sequences which are located downstream of said nucleic acid sequence.

Promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression and/or to alter the spatial expression and/or temporal expression of a nucleic acid molecule to which it is operably connected. Such regulatory elements may be placed adjacent to a heterologous promoter sequence to drive expression of a nucleic acid molecule in response to e.g. copper, glucocorticoids, dexamethasone, tetracycline, gibberellin, cAMP, abscisic acid, auxin, wounding, ethylene, jasmonate or salicylic acid or to confer expression of a nucleic acid molecule to specific cells, tissues or organs such as meristems, leaves, roots, embryo, flowers, seeds or fruits.

In the context of the present invention, the promoter preferably is a plant-expressible promoter sequence. Promoters, however, that also function or solely function in non-plant cells such as bacteria, yeast cells, insect cells and animal cells are not excluded from the invention. By "plant-expressible" is meant that the promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ.

The terms "plant-operable" and "operable in a plant" when used herein, in respect of a promoter sequence, shall be taken to be equivalent to a plant-expressible promoter sequence.

In the present context, a "regulatable promoter sequence" is a promoter that is capable of conferring expression on a structural gene in a particular cell, tissue, or organ or group of cells, tissues or organs of a plant, optionally under specific conditions, however does generally not confer expression throughout the plant under all conditions. Accordingly, a regulatable promoter sequence may be a promoter sequence that confers expression on a gene to which it is operably connected in a particular location within the plant or alternatively, throughout the plant under a specific set of conditions, such as following induction of gene expression by a chemical compound or other elicitor.

Preferably, the regulatable promoter used in the performance of the present invention confers expression in a specific location within the plant, either constitutively or following induction, however not in the whole plant under any circumstances. Included within the scope of such promoters are cell-specific promoter sequences, tissue-specific promoter

sequences, organ-specific promoter sequences, cell cycle specific gene promoter sequences, inducible promoter sequences and constitutive promoter sequences that have been modified to confer expression in a particular part of the plant at any one time, such as by integration of said constitutive promoter within a transposable genetic element (*Ac*, *Ds*, *Spm*, *En*, or other transposon). The skilled craftsman will understand that a "constitutive promoter" is a promoter that is transcriptionally active in an organism, preferably a plant, during most, but not necessarily all phases of its growth and development. Similarly, the skilled craftsman will understand that a "ubiquitous promoter" is a promoter that is transcriptionally active throughout most, but not necessarily all parts of an organism, preferably a plant.

Generally by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts, to about 1/500,000 transcripts. Conversely, a "strong promoter" drives expression of a coding sequence at high level, or at about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

The term "cell-specific" shall be taken to indicate that expression is predominantly in a particular cell or cell-type, preferably of plant origin, albeit not necessarily exclusively in said cell or cell-type.

Similarly, the term "tissue-specific" shall be taken to indicate that expression is predominantly in a particular tissue or tissue-type, preferably of plant origin, albeit not necessarily exclusively in said tissue or tissue-type.

Similarly, the term "organ-specific" shall be taken to indicate that expression is predominantly in a particular organ, preferably of plant origin, albeit not necessarily exclusively in said organ. "Root specific" means that the promoter is expressed in the root only and not in other tissues of the plant.

By "root-preferred" it is intended that expression of the heterologous nucleotide sequence is most abundant root, but could also have low expression levels elsewhere in the plant. While some level of expression of the heterologous nucleotide sequence occurs in other plant tissue types, expression occurs most abundantly in the root including primary, lateral and adventitious roots.

By "root" is intended any part of the root structure, including, but not limited to, the root cap, apical meristem, protoderm, ground meristem, procambium, endodermis, cortex, vascular cortex, epidermis, and the like.

Similarly, the term "cell cycle specific" shall be taken to indicate that expression is predominantly cyclic and occurring in one or more, not necessarily consecutive phases of the cell cycle albeit not necessarily exclusively in cycling cells, preferably of plant origin.

5 Contrarily, the term ubiquitous shall be taken to indicate that expression is throughout the entire organism.

Those skilled in the art will be aware that an "inducible promoter" is a promoter the transcriptional activity of which is increased or induced in response to a developmental, chemical, environmental, or physical stimulus. Similarly, the skilled craftsman will  
10 understand that a "constitutive promoter" is a promoter that is transcriptionally active throughout most, but not necessarily all parts of an organism, preferably a plant, during most, but not necessarily all phases of its growth and development.

Those skilled in the art will readily be capable of selecting appropriate promoter sequences for use in regulating appropriate expression of the LRR receptor-like kinase  
15 as described supra from publicly-available or readily-available sources, without undue experimentation.

Placing a nucleic acid molecule under the regulatory control of a promoter sequence, or in operable connection with a promoter sequence, means positioning said nucleic acid molecule such that expression is controlled by the promoter sequence. A promoter is  
20 usually, but not necessarily, positioned upstream, or at the 5'-end, and within 2 kb of the start site of transcription, of the nucleic acid molecule which it regulates. In the construction of heterologous promoter/structural gene combinations it is generally preferred to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it  
25 controls in its natural setting (i.e., the gene from which the promoter is derived). As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting (i.e., the gene from which it is derived).  
30 Again, as is known in the art, some variation in this distance can also occur.

"Expression" means the production of a protein or nucleotide sequence in the cell itself or in a cell-free system. It includes transcription into an RNA product, post-transcriptional

modification and/or translation to a protein product or polypeptide from a DNA encoding that product, as well as possible post-translational modifications.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence  
5 "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used.

Examples of promoters suitable for use in gene constructs of the present invention  
10 include those listed in Table 3, amongst others. The promoters listed in Table 5 are provided for the purposes of exemplification only and the present invention is not to be limited by the list provided therein. Those skilled in the art will readily be in a position to provide additional promoters that are useful in performing the present invention.

In the case of constitutive promoters or promoters that induce expression throughout the  
15 entire plant, it is preferred that such sequences are modified by the addition of nucleotide sequences derived from one or more of the tissue-specific promoters listed in Table 8, or alternatively, nucleotide sequences derived from one or more of the above-mentioned tissue-specific inducible promoters, to confer tissue-specificity thereon. For example, the CaMV 35S promoter may be modified by the addition of maize *Adh1*  
20 promoter sequence, to confer anaerobically-regulated root-specific expression thereon, as described previously (Ellis *et al.*, 1987). Another example describes conferring root specific or root abundant gene expression by fusing the CaMV35S promoter to elements of the maize glycine-rich protein GRP3 gene (Feix and Wulff 2000 - WO0015662). Such modifications can be achieved by routine experimentation by those skilled in the art.

25 The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in cells derived from viruses, yeasts, moulds, bacteria, insects, birds, mammals and plants are known and  
30 described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the gene constructs of the present invention include the *Agrobacterium tumefaciens* nopaline synthase (NOS) gene

terminator, the *Agrobacterium tumefaciens* octopine synthase (OCS) gene terminator sequence, the Cauliflower mosaic virus (CaMV) 35S gene terminator sequence, the *Oryza sativa* ADP-glucose pyrophosphorylase terminator sequence (t3'Bt2), the *Zea mays* zein gene terminator sequence, the *rbcS-1A* gene terminator, and the *rbcS-3A* gene terminator sequences, amongst others.

**Table 3. Exemplary plant-expressible promoters for use  
in the performance of the present invention**

<b>I: CELL-SPECIFIC, TISSUE-SPECIFIC, AND ORGAN-SPECIFIC PROMOTERS</b>		
<b>GENE SOURCE</b>	<b>EXPRESSION PATTERN</b>	<b>REFERENCE</b>
$\alpha$ -amylase ( <i>Amy32b</i> )	Aleurone	Lanahan et al, Plant Cell 4:203-211, 1992; Skriver et al, Proc Natl Acad Sci USA 88:7266-7270, 1991
cathepsin $\beta$ -like gene	Aleurone	Cejudo et al, Plant Mol Biol 20:849-856, 1992
<i>Agrobacterium</i> <i>rhizogenes rolB</i>	Cambium	Nilsson et al, Physiol Plant 100:456-462, 1997
AtPRP4	Flowers	<a href="http://salus.medium.edu/mmg/tierney/html">http://salus.medium.edu/mmg/tierney/html</a>
chalcone synthase ( <i>chsA</i> )	Flowers	Van der Meer et al, Plant Mol Biol 15:95- 109, 1990
LAT52	Anther	Twell et al, Mol Gen Genet 217:240-245, 1989
<i>apetala-3</i>	Flowers	
chitinase	fruit (berries, grapes, etc)	Thomas et al. CSIRO Plant Industry, Urrbrae, South Australia, Australia; <a href="http://winetitles.com.au/gwrdc/csh95-1.html">http://winetitles.com.au/gwrdc/csh95-1.html</a>
rbcS-3A	green tissue (eg leaf)	Lam et al, Plant Cell 2:857-866, 1990; Tucker et al., Plant Physiol 113:1303-1308, 1992
leaf-specific genes	Leaf	Baszczynski et al, Nucl Acid Res 16:4732, 1988
AtPRP4	Leaf	<a href="http://salus.medium.edu/mmg/tierney/html">http://salus.medium.edu/mmg/tierney/html</a>
chlorella virus adenine methyltransferase gene promoter	Leaf	Mitra and Higgins, Plant Mol Biol 26:85-93, 1994
aldP gene promoter from rice	Leaf	Kagaya et al, Mol Gen Genet 248:668-674, 1995

rbcs promoter from rice or tomato	Leaf	Kyozuka et al, Plant Physiol 102:991-1000, 1993
<i>Pinus cab-6</i>	Leaf	Yamamoto et al, Plant Cell Physiol 35:773-778, 1994
rubisco promoter	Leaf	
<i>cab</i> (chlorophyll a/b/binding protein)	Leaf	
pea Blec4 gene	Vegetative and floral epidermal tissues	Mandaci and Dobres, Plant Mol Biol 34:961-965
SAM22	senescent leaf	Crowell et al, Plant Mol Biol 18:459-466, 1992
<i>ltp</i> gene (lipid transfer gene)		Fleming et al, Plant J 2:855-862, 1992
<i>R. japonicum nif</i> gene	nodule	United States Patent No 4 803165
<i>B. japonicum nifH</i> gene	nodule	United States Patent No 5008194
GmENOD40	nodule	Yang et al, Plant J 3:573-585, 1993
PEP carboxylase (PEPC)	nodule	Pathirana et al, Plant Mol Biol 20:437-450, 1992
Leghaemoglobin (Lb)	nodule	Gordon et al, J Exp Bot 44:1453-1465, 1993
<i>Tungro bacilliform</i> virus gene	phloem	Bhattacharyya-Pakrasi et al, Plant J 4:71-79, 1992
pollen-specific genes	pollen; microspore	Albani et al, Plant Mol Biol 15:605, 1990; Albani et al, Plant Mol Biol 16:501, 1991
Zm13	pollen	Guerrero et al, Mol Gen Genet 224:161-168, 1993
apg gene	microspore	Twell et al, Sex Plant Reprod 6:217-224, 1993
maize pollen-specific gene	pollen	Hamilton et al, Plant Mol Biol 18:211-218, 1992
Sunflower pollen-expressed gene	pollen	Baltz et al, Plant J 2:713-721, 1992
<i>B. napus</i> pollen-	pollen; anther; tapetum	Arnoldo et al, J Cell Biochem, Abstract No.

specific gene		Y101, 204, 1992
root-expressible genes	roots	Tingey et al, EMBO J 6:1, 1987
Tobacco auxin-inducible gene	root tip	Van der Zaal et al, Plant Mol Biol 16:983, 1991
$\beta$ -tubulin	root	Oppenheimer et al, Gene 63:87, 1988
Tobacco root-specific genes	root	Conkling et al, Plant Physiol 93:1203, 1990
<i>B. napus</i> G1-3b gene	root	United States Patent No 5401836
SbPRP1	roots	Suzuki et al, Plant Mol Biol 21:109-119, 1993
AtPRP1; AtPRP3	roots; root hairs	<a href="http://salus.medium.edu/mmg/tierney/html">http://salus.medium.edu/mmg/tierney/html</a>
RD2 gene	root cortex	<a href="http://www2.cnsu.edu/ncsu/research">http://www2.cnsu.edu/ncsu/research</a>
TobRB7 gene	root vasculature	<a href="http://www2.cnsu.edu/ncsu/research">http://www2.cnsu.edu/ncsu/research</a>
AtPRP4	leaves; flowers; lateral root primordia	<a href="http://salus.medium.edu/mmg/tierney/html">http://salus.medium.edu/mmg/tierney/html</a>
seed-specific genes	seed	Simon et al, Plant Mol Biol 5:191, 1985; Scofield et al, J Biol Chem 262:12202, 1987; Baszczynski et al, Plant Mol Biol 14:633, 1990
Brazil Nut albumin	seed	Pearson et al, Plant Mol Biol 18:235-245, 1992
Legumin	seed	Ellis et al, Plant Mol Biol 10:203-214, 1988
Glutelin (rice)	seed	Takaiwa et al, Mol Gen Genet 208:15-22, 1986; Takaiwa et al, FEBS Lett 221:43-47, 1987
Zein	seed	Matzke et al, Plant Mol Biol 14:323-32 1990
NapA	seed	Stalberg et al, Planta 199:515-519, 1996
wheat LMW and HMW glutenin-1	endosperm	Mol Gen Genet 216:81-90, 1989; Nucl Acids Res 17:461-462, 1989
wheat SPA	seed	Albani et al, Plant Cell 9:171-184, 1997
cZ19B1, maize 19 kDa zein	seed	WO0011177
mi1ps, maize	seed	WO0011177



myoinositol-1-Pi synthase		
wheat $\alpha$ , $\beta$ , $\gamma$ -gliadins	endosperm	EMBO J 3:1409-1415, 1984
barley <i>ltr1</i> promoter	endosperm	
barley B1, C, D, hordein	endosperm	Theor Appl Gen 98:1253-1262, 1999; Plant J 4:343-355, 1993; Mol Gen Genet 250:750-60, 1996
barley DOF	endosperm	Mena et al, Plant J 116:53-62, 1998
<i>blz2</i>	endosperm	EP99106056.7
synthetic promoter	endosperm	Vicente-Carbajosa et al, Plant J 13:629-640, 1998
rice prolamin NRP33	endosperm	Wu et al, Plant Cell Physiol 39: 885-889, 1998
rice $\alpha$ -globulin G1b-1	endosperm	Wu et al, Plant Cell Physiol 39:885-889, 1998
maize END genes	endosperm	WO0012733
barley END1	endosperm	WO9808961
barley NUC1	nucellus	WO9808961
rice OSH1	embryo	Sato et al, Proc Natl Acad Sci USA 93:8117-8122, 1996
rice $\alpha$ -globulin REB/OHP-1	endosperm	Nakase et al, Plant Mol Biol 33:513-522, 1997
rice ADP-glucose PP	endosperm	Trans Res 6:157-168, 1997
maize ESR gene family	endosperm	Plant J 12:235-246, 1997
sorgum $\gamma$ -kafirin	endosperm	Plant Mol Biol 32:1029-1035, 1996
KNOX	embryo	Postma-Haarsma et al, Plant Mol Biol 39:257-271, 1999
rice oleosin	embryo and aleuron	Wu et al, J Biochem 123:386, 1998
sunflower oleosin	seed (embryo and dry seed)	Cummins et al, Plant Mol Biol 19:873-876, 1992
<i>LEAFY</i>	shoot meristem	Weigel et al, Cell 69:843-859, 1992
<i>Arabidopsis thaliana knat1</i>	shoot meristem	Accession number AJ131822
<i>Malus domestica</i>	shoot meristem	Accession number Z71981

<i>kn1</i>		
<i>CLAVATA1</i>	shoot meristem	Accession number AF049870
stigma-specific genes	stigma	Nasrallah et al, Proc Natl Acad Sci USA 85:5551, 1988; Trick et al, Plant Mol Biol 15:203, 1990
class I patatin gene	tuber	Liu et al, Plant Mol Biol 153:386-395, 1991
PCNA rice	meristem	Kosugi et al, Nucl Acids Res 19:1571-1576, 1991; Kosugi and Ohashi, Plant Cell 9:1607-1619, 1997
Pea TubA1 tubulin	Dividing cells	Stotz and Long, Plant Mol Biol 41:601-614, 1999
<i>Arabidopsis cdc2a</i>	cycling cells	Chung and Parish, FEBS Lett 362:215-219, 1995
<i>Arabidopsis Rop1A</i>	Anthers; mature pollen + pollen tubes	Li et al, Plant Physiol 118:407-417, 1998
<i>Arabidopsis AtDMC1</i>	Meiosis-associated	Klimyuk and Jones, Plant J 11:1-14, 1997
Pea PS-IAA4/5 and PS-IAA6	Auxin-inducible	Wong et al, Plant J 9:587-599, 1996
Pea farnesyltransferase	Meristematic tissues; phloem near growing tissues; light- and sugar-repressed	Zhou et al, Plant J 12:921-930, 1997
Tobacco ( <i>N. sylvestris</i> ) cyclin B1;1	Dividing cells / meristematic tissue	Trehin et al, Plant Mol.Biol. 35:667-672, 1997
<i>Catharanthus roseus</i> Mitotic cyclins CYS (A-type) and CYM (B-type)	Dividing cells / meristematic tissue	Ito et al, Plant J 11:983-992, 1997
<i>Arabidopsis cyc1At</i> (=cyc B1;1) and <i>cyc3aAt</i> (A-type)	Dividing cells / meristematic tissue	Shaul et al, Proc Natl Acad Sci USA 93:4868-4872, 1996
<i>Arabidopsis tef1</i> promoter box	Dividing cells / meristematic tissue	Regad et al, Mol Gen Genet 248:703-711, 1995
<i>Catharanthus roseus</i>	Dividing cells /	Ito et al, Plant Mol Biol 24:863-878, 1994

cyc07	meristematic tissue	
<b>II: EXEMPLARY CONSTITUTIVE PROMOTERS</b>		
<b>GENE SOURCE</b>	<b>EXPRESSION PATTERN</b>	<b>REFERENCE</b>
Actin	constitutive	McElroy et al, Plant Cell 2:163-171, 1990
CAMV 35S	constitutive	Odell et al, Nature 313:810-812, 1985
CaMV 19S	constitutive	Nilsson et al, Physiol Plant 100:456-462, 1997
GOS2	constitutive	de Pater et al, Plant J 2:837-844, 1992
Ubiquitin	constitutive	Christensen et al, Plant Mol Biol 18:675-689, 1992
rice cyclophilin	constitutive	Buchholz et al, Plant Mol Biol 25:837-843, 1994
maize histone H3	constitutive	Lepetit et al, Mol Gen Genet 231:276-285, 1992
alfalfa histone H3	constitutive	Wu et al, Nucleic Acids Res 17:3057-3063, 1989; Wu et al, Plant Mol Biol 11:641-649, 1988
actin 2	constitutive	An et al, Plant J 10:107-121, 1996
<b>III: EXEMPLARY STRESS-INDUCIBLE PROMOTERS</b>		
<b>NAME</b>	<b>STRESS</b>	<b>REFERENCE</b>
P5CS (delta(1)-pyrroline-5-carboxylate syntase)	salt, water	Zhang et al, Plant Sci 129:81-89, 1997
cor15a	cold	Hajela et al, Plant Physiol 93:1246-1252, 1990
cor15b	cold	Wiihelm et al, Plant Mol Biol 23:1073-1077, 1993
cor15a (-305 to +78 nt)	cold, drought	Baker et al, Plant Mol Biol 24: 01-713, 1994
rd29	salt, drought, cold	Kasuga et al, Nature Biotechnol 18:287-291, 1999
heat shock proteins,	heat	Barros et al, Plant Mol Biol 19 665-75,

including artificial promoters containing the heat shock element (HSE)		1992. Marrs et al, Dev Genet 14:27-41, 1993. Schoffl et al, Mol Gen Genet 217:246-53, 1989.
SmHSP (small heat shock proteins)	heat	Waters et al, J Exp Bot 47:325-338, 1996
wcs120	cold	Ouellete et al, FEBS Lett 423:324-328, 1998
ci7	cold	Kirch et al, Plant Mol Biol 33:897-909, 1997
Adh	cold, drought, hypoxia	Dolferus et al, Plant Physiol 105:1075-87, 1994
pws18	salt and drought	Joshee et al, Plant Cell Physiol 39:64-72, 1998
ci21A	cold	Schneider et al, Plant Physiol 113:335-45, 1997
Trg-31	drought	Chaudhary et al, Plant Mol Biol 30:1247-57, 1996
Osmotin	osmotic	Raghothama et al, Plant Mol Biol 23:1117-28, 1993
LapA	wounding, enviromental	WO99/03977 University of California/INRA

#### IV: EXEMPLARY PATHOGEN-INDUCIBLE PROMOTERS

NAME	PATHOGEN	REFERENCE
RB7	Root-knot nematodes (Meloidogyne spp.)	US5760386 - North Carolina State University; Opperman et al, Science 263:221-23, 1994
PR-1, 2, 3, 4, 5, 8, 11	fungal, viral, bacterial	Ward et al, Plant Cell 3:1085-1094, 1991; Reiss et al 1996; Lebel et al, Plant J 16:223-233, 1998; Melchers et al, Plant J 5:469-480, 1994; Lawton et al, Plant Mol Biol, 19:735-743, 1992
HMG2	nematodes	WO9503690 - Virginia Tech Intellectual Properties Inc .
Abi3	Cyst nematodes	unpublished

	(Heterodera spp.)	
ARM1	nematodes	Barthels et al, Plant Cell 9:2119-2134, 1997 WO 98/31822 – Plant Genetic Systems
Att0728	nematodes	Barthels et al, Plant Cell 9: 2119-2134, 1997 PCT/EP98/07761
Att1712	nematodes	Barthels et al, Plant Cell 9, 2119-2134, 1997 PCT/EP98/07761
Gst1	Different types of pathogens	Strittmatter et al, Mol Plant-Microbe Interact 9:68-73, 1996
LEMMI	nematodes	WO 92/21757 – Plant Genetic Systems
CLE	geminivirus	PCT/EP99/03445 - CINESTAV
PDF1.2	Fungal including <i>Alternaria brassicicola</i> and <i>Botrytis cinerea</i>	Manners et al, Plant Mol Biol, 38:1071- 1080, 1998
Thi2.1	Fungal – <i>Fusarium</i> <i>oxysporum</i> f sp. <i>matthiolae</i>	Vignutelli et al, Plant J 14:285-295, 1998
DB#226	nematodes	Bird and Wilson, Mol Plant-Microbe Interact 7:419-442, 1994 WO 95.322888
DB#280	nematodes	Bird and Wilson, Mol Plant-Microbe Interact 7:419-442, 1994 WO 95.322888
Cat2	nematodes	Niebel et al, Mol Plant-Microbe Interact 8:371-378, 1995
□Tub	nematodes	Aristizabal et al (1996), 8 <sup>th</sup> International Congress on Plant-Microbe Interaction, Knoxville US B-29
SHSP	nematodes	Fenoll et al (1997) In: Cellular and molecular aspects of plant-nematode interactions. Kluwer Academic, C. Fenoll, F.M.W. Grundler and S.A. Ohi (Eds.),
Tsw12	nematodes	Fenoll et al (1997) In: Cellular and

		molecular aspects of plant-nematode interactions. Kluwer Academic, C. Fenoll, F.M.W. Grundler and S.A. Ohi (Eds.)
Hs1(pro1)	nematodes	WO 98/122335 - Jung
NsLTP	viral, fungal, bacterial	Molina and Garcia-Olmedo FEBS Lett, 316:119-122, 1993
RIP	viral, fungal	Turner et al, Proc Natl Acad Sci USA 94:3866-3871, 1997

Those skilled in the art will be aware of additional promoter sequences and terminator sequences which may be suitable for use in performing the invention. Such sequences may readily be used without any undue experimentation.

- 5 In the context of the current invention, "ectopic expression" or "ectopic overexpression" of a gene or a protein are conferring to expression patterns and/or expression levels of said gene or protein normally not occurring under natural conditions. Ectopic expression can be achieved in a number of ways including operably linking of a coding sequence encoding said protein to an isolated homologous or heterologous promoter in order to
- 10 create a chimeric gene and/or operably linking said coding sequence to its own isolated promoter (i.e. the unisolated promoter naturally driving expression of said protein) in order to create a recombinant gene duplication or gene multiplication effect. With "ectopic co-expression" is meant the ectopic expression or ectopic overexpression of two or more genes or proteins. The same or, more preferably, different promoters are used
- 15 to confer expression of said genes or proteins.

Preferably, the promoter sequence used in the context of the present invention is operably linked to a coding sequence or open reading frame (ORF) encoding a LRR receptor-like kinase or a homolog, derivative and/or an immunologically active fragment thereof as defined supra.

- 20 "Dominant negative version or variant" refers to a mutant protein, which interferes with the activity of the corresponding wild-type protein.

"Downregulation of expression" as used herein means lowering levels of gene expression and/or levels of active gene product and/or levels of gene product activity. Decreases in expression may be accomplished by e.g. the addition of coding sequences

25 or parts thereof in a sense orientation (if resulting in co-suppression) or in an antisense

orientation relative to a promoter sequence and furthermore by e.g. insertion mutagenesis (e.g. T-DNA insertion or transposon insertion) or by gene silencing strategies as described by e.g. Angell and Baulcombe (1998 - WO9836083), Lowe et al. (1989 - WO9853083), Lederer et al. (1999 - WO9915682) or Wang et al. (1999 - WO9953050). Genetic constructs aimed at silencing gene expression may have the nucleotide sequence of said gene (or one or more parts thereof) contained therein in a sense and/or antisense orientation relative to the promoter sequence. Another method to downregulate gene expression comprises the use of ribozymes, e.g. as described in Atkins et al. 1994 (WO9400012), Lenée et al. 1995 (WO9503404), Lutziger et al. 2000 (WO0000619), Prinsen et al. 1997 (WO9713865) and Scott et al. 1997 (WO9738116).

Modulating, including lowering, the level of active gene products or of gene product activity can be achieved by administering or exposing cells, tissues, organs or organisms to said gene product, a homolog, analogue, derivative and/or immunologically active fragment thereof. Immunomodulation is another example of a technique capable of downregulation levels of active gene product and/or of gene product activity and comprises administration of or exposing to or expressing antibodies to said gene product to or in cells, tissues, organs or organisms wherein levels of said gene product and/or gene product activity are to be modulated. Such antibodies comprise "plantibodies", single chain antibodies, IgG antibodies and heavy chain camel antibodies as well as fragments thereof.

Modulating, including lowering, the level of active gene products or of gene product activity can furthermore be achieved by administering or exposing cells, tissues, organs or organisms to an inhibitor or activator of said gene product or the activity thereof. Such inhibitors or activators include proteins (comprising e.g. proteinases and kinases) and chemical compounds identified according to the current invention as described supra.

In the context of the invention the term "agonist" refers to a substance that can be either a protagonist or an antagonist, i.e. can have either positive or negative effects, can be an enhancer or an inhibitor or a modulator as well.

In the context of the current invention is envisaged the downregulation of the expression of a LRR receptor-like kinase gene as defined higher. The invention further comprises downregulation of levels of a LRR receptor-like kinase or of a LRR receptor-like kinase activity whereby said LRR receptor-like kinase has been defined supra.

By "cell fate and/or plant development and/or plant morphology and/or biochemistry and/or physiology" is meant that one or more developmental and/or morphological and/or biochemical and/or physiological characteristics of a plant is altered by the performance of one or more steps pertaining to the invention described herein.

5 "Cell fate" refers to the cell-type or cellular characteristics of a particular cell that are produced during plant development or a cellular process therefor, in particular during the cell cycle or as a consequence of a cell cycle process.

"Plant development" or the term "plant developmental characteristic" or similar term shall, when used herein, be taken to mean any cellular process of a plant that is involved  
10 in determining the developmental fate of a plant cell, in particular the specific tissue or organ type into which a progenitor cell will develop. Cellular processes relevant to plant development will be known to those skilled in the art. Such processes include, for example, morphogenesis, photomorphogenesis, shoot development, root development, vegetative development, reproductive development, stem elongation, flowering, and  
15 regulatory mechanisms involved in determining cell fate, in particular a process or regulatory process involving the cell cycle.

"Plant morphology" or the term "plant morphological characteristic" or similar term will, when used herein, be understood by those skilled in the art to refer to the external appearance of a plant, including any one or more structural features or combination of  
20 structural features thereof. Such structural features include the shape, size, number, position, colour, texture, arrangement, and patternation of any cell, tissue or organ or groups of cells, tissues or organs of a plant, including the root, stem, leaf, shoot, petiole, trichome, flower, petal, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, fruit, cambium, wood, heartwood, parenchyma, aerenchyma,  
25 sieve element, phloem or vascular tissue, amongst others.

"Plant biochemistry" or the term "plant biochemical characteristic" or similar term will, when used herein, be understood by those skilled in the art to refer to the metabolic and catalytic processes of a plant, including primary and secondary metabolism and the products thereof, including any small molecules, macromolecules or chemical  
30 compounds, such as but not limited to starches, sugars, proteins, peptides, enzymes, hormones, growth factors, nucleic acid molecules, celluloses, hemicelluloses, calloses, lectins, fibres, pigments such as anthocyanins, vitamins, minerals, micronutrients, or macronutrients, that are produced by plants.



"Plant physiology" or the term "plant physiological characteristic" or similar term will, when used herein, be understood to refer to the functional processes of a plant, including developmental processes such as growth, expansion and differentiation, sexual development, sexual reproduction, seed set, seed development, grain filling, asexual reproduction, cell division, dormancy, germination, light adaptation, photosynthesis, leaf expansion, fiber production, secondary growth or wood production, amongst others; responses of a plant to externally-applied factors such as metals, chemicals, hormones, growth factors, environment and environmental stress factors (eg. anoxia, hypoxia, high temperature, low temperature, dehydration, light, daylength, flooding, salt, heavy metals, amongst others), including adaptive responses of plants to said externally-applied factors.

Means for introducing recombinant DNA into plant tissue or cells include, but are not limited to, transformation using  $\text{CaCl}_2$  and variations thereof, in particular the method described by Hanahan (1983), direct DNA uptake into protoplasts (Krens *et al.*, 1982; Paszkowski *et al.*, 1984), PEG-mediated uptake to protoplasts (Armstrong *et al.*, 1990) microparticle bombardment, electroporation (Fromm *et al.*, 1985), microinjection of DNA (Crossway *et al.*, 1986), microparticle bombardment of tissue explants or cells (Christou *et al.*, 1988), vacuum-infiltration of tissue with nucleic acid, or in the case of plants, T-DNA-mediated transfer from *Agrobacterium* to the plant tissue as described essentially by An *et al.* (1985), Dodds *et al.* (1985), Herrera-Estrella *et al.* (1983a, 1983b). Methods for transformation of monocotyledonous plants are well known in the art and include *Agrobacterium*-mediated transformation (Cheng *et al.* 1997 - WO9748814; Hansen 1998 - WO9854961, Hiei *et al.* 1994 - WO9400977; Hiei *et al.* 1998 - WO9817813; Rikiishi *et al.* 1999 - WO9904618; Saito *et al.* 1995 - WO9506722), microprojectile bombardment (Adams *et al.* 1999 - US5969213; Bowen *et al.* 1998 - US5736369; Chang *et al.* 1994 - WO9413822; Lundquist *et al.* 1999 - US5874265/US5990390; Vasil and Vasil 1995 - US5405765; Walker *et al.* 1999 - US5955362), DNA uptake (Eyal *et al.* 1993 - WO9318168), microinjection of *Agrobacterium* cells (von Holt 1994 - DE4309203) and sonication (Finer *et al.* 1997 - US5693512).

For microparticle bombardment of cells, a microparticle is propelled into a cell to produce a transformed cell. Any suitable ballistic cell transformation methodology and apparatus can be used in performing the present invention. Exemplary apparatus and procedures are disclosed by Stomp *et al.* (U.S. Patent No. 5122466) and Sanford and Wolf (U.S.

Patent No. 4945050). When using ballistic transformation procedures, the gene construct may incorporate a plasmid capable of replicating in the cell to be transformed. Examples of microparticles suitable for use in such systems include 1 to 5  $\mu\text{m}$  gold spheres. The DNA construct may be deposited on the microparticle by any suitable  
5 technique, such as by precipitation.

A whole plant may be regenerated from the transformed or transfected cell, in accordance with procedures well known in the art. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a gene construct of the present invention and a whole plant regenerated therefrom.

10 The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon  
15 meristem and hypocotyl meristem).

The term "organogenesis", as used herein, means a process by which shoots and roots are developed sequentially from meristematic centers.

The term "embryogenesis", as used herein, means a process by which shoots and roots develop together in a concerted fashion (not sequentially), whether from somatic cells or  
20 gametes.

Preferably, the plant is produced according to the inventive method is transfected or transformed with a genetic sequence, or amenable to the introduction of a protein, by any art-recognized means, such as microprojectile bombardment, microinjection, *Agrobacterium*-mediated transformation (including the 'flower dip' transformation  
25 method; Bechtold and Pelletier 1998, Trieu et al. 2000), protoplast fusion, or electroporation, amongst others. Most preferably said plant is produced by *Agrobacterium*-mediated transformation.

The "seedling" is the juvenile plant that arises from the mature embryo after seed germination.

30 With "differentiation of a cell" it is understood that the cell develops unique features to be engaged for a specific function. Mostly differentiation is irreversible.

*Agrobacterium*-mediated transformation or agrolistic transformation of plants, yeast, moulds or filamentous fungi is based on the transfer of part of the transformation vector

sequences, called the T-DNA, to the nucleus and on integration of said T-DNA in the genome of said eukaryote.

With "*Agrobacterium*" is meant a member of the *Agrobacteriaceae*, more preferably *Agrobacterium* or *Rhizobacterium* and most preferably *Agrobacterium tumefaciens*.

5 With "T-DNA", or transferred DNA, is meant that part of the transformation vector flanked by T-DNA borders which is, after activation of the *Agrobacterium vir* genes, nicked at the T-DNA borders and is transferred as a single stranded DNA to the nucleus of an eukaryotic cell.

When used herein, with "T-DNA borders", "T-DNA border region", or "border region" are  
10 meant either right T-DNA border (RB) or left T-DNA border (LB). Such a border comprises a core sequence flanked by a border inner region as part of the T-DNA flanking the border and/or a border outer region as part of the vector backbone flanking the border. The core sequences comprise 22 bp in case of octopine-type vectors and 25 bp in case of nopaline-type vectors. The core sequences in the right border region and  
15 left border region form imperfect repeats. Border core sequences are indispensable for recognition and processing by the *Agrobacterium* nicking complex consisting of at least VirD1 and VirD2. Core sequences flanking a T-DNA are sufficient to promote transfer of said T-DNA. However, efficiency of transformation using transformation vectors carrying said T-DNA solely flanked by said core sequences is low. Border inner and outer regions  
20 are known to modulate efficiency of T-DNA transfer (Wang et al. 1987). One element enhancing T-DNA transfer has been characterized and resides in the right border outer region and is called *overdrive* (Peralta et al. 1986, van Haaren et al. 1987).

With "T-DNA transformation vector" or "T-DNA vector" is meant any vector encompassing a T-DNA sequence flanked by a right and left T-DNA border consisting of  
25 at least the right and left border core sequences, respectively, and used for transformation of any eukaryotic cell.

With "T-DNA vector backbone sequence" or "T-DNA vector backbone sequences" is meant all DNA of a T-DNA containing vector that lies outside of the T-DNA borders and, more specifically, outside the nicking sites of the border core imperfect repeats.

30 The current invention includes optimized T-DNA vectors such that vector backbone integration in the genome of a eukaryotic cell is minimized or absent. With "optimized T-DNA vector" is meant a T-DNA vector designed either to decrease or abolish transfer of vector backbone sequences to the genome of a eukaryotic cell. Such T-DNA vectors are

known to the one familiar with the art and include those described by Hanson et al. (1999) and by Stuiver et al. (1999 - WO9901563).

The current invention clearly considers the inclusion of a DNA sequence comprising the promoter sequence of the present invention encoding a LRR receptor-like kinase, homolog, derivative or immunologically active fragment thereof as defined supra, in any T-DNA vector comprising binary transformation vectors, super-binary transformation vectors, co-integrate transformation vectors, Ri-derived transformation vectors as well as in T-DNA carrying vectors used in agrolistic transformation.

With "binary transformation vector" is meant a T-DNA transformation vector comprising:

a T-DNA region comprising at least one gene of interest and/or at least one selectable marker active in the eukaryotic cell to be transformed; and

a vector backbone region comprising at least origins of replication active in *E. coli* and *Agrobacterium* and markers for selection in *E. coli* and *Agrobacterium*.

The T-DNA borders of a binary transformation vector can be derived from octopine-type or nopaline-type Ti plasmids or from both. The T-DNA of a binary vector is only transferred to a eukaryotic cell in conjunction with a helper plasmid. Also known in the art are multiple binary vector *Agrobacterium* strains for efficient co-transformation of plants (Bidney and Scelongo 2000 - WO0018939).

With "helper plasmid" is meant a plasmid that is stably maintained in *Agrobacterium* and is at least carrying the set of *vir* genes necessary for enabling transfer of the T-DNA. Said set of *vir* genes can be derived from either octopine-type or nopaline-type Ti plasmids or from both.

With "super-binary transformation vector" is meant a binary transformation vector additionally carrying in the vector backbone region a *vir* region of the Ti plasmid pTiBo542 of the super-virulent *A. tumefaciens* strain A281 (Hiei et al. 1994 - EP0604662, Hiei et al. 1995 - EP0687730). Super-binary transformation vectors are used in conjunction with a helper plasmid.

With "co-integrate transformation vector" is meant a T-DNA vector at least comprising:

a T-DNA region comprising at least one gene of interest and/or at least one selectable marker active in plants; and

a vector backbone region comprising at least origins of replication active in *Escherichia coli* and *Agrobacterium*, and markers for selection in *E. coli* and *Agrobacterium*, and a set of *vir* genes necessary for enabling transfer of the T-DNA.

The T-DNA borders and said set of *vir* genes of a said T-DNA vector can be derived from either octopine-type or nopaline-type Ti plasmids or from both.

With "Ri-derived plant transformation vector" is meant a binary transformation vector in which the T-DNA borders are derived from a Ti plasmid and said binary transformation vector being used in conjunction with a 'helper' Ri-plasmid carrying the necessary set of *vir* genes.

As used herein, the term "selectable marker gene" or "selectable marker" or "marker for selection" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a gene construct of the invention or a derivative thereof. Suitable selectable marker genes contemplated herein include the ampicillin resistance (*Amp<sup>r</sup>*), tetracycline resistance gene (*Tc<sup>r</sup>*), bacterial kanamycin resistance gene (*Kan<sup>r</sup>*), phosphinothricin resistance gene, neomycin phosphotransferase gene (*nptII*), hygromycin resistance gene,  $\beta$ -glucuronidase (GUS) gene, chloramphenicol acetyltransferase (CAT) gene, green fluorescent protein (*gfp*) gene (Haseloff *et al*, 1997), and luciferase gene, amongst others.

With "agrolistics", "agrolistic transformation" or "agrolistic transfer" is meant here a transformation method combining features of *Agrobacterium*-mediated transformation and of biolistic DNA delivery. As such, a T-DNA containing target plasmid is co-delivered with DNA/RNA enabling in planta production of VirD1 and VirD2 with or without VirE2 (Hansen and Chilton 1996; Hansen *et al.* 1997; Hansen and Chilton 1997 - WO9712046). The present invention further describes an approach to remove from transformed cells a stably integrated foreign DNA sequence by recombination involving a recombinase and recombination sites.

With "foreign DNA" is meant any DNA sequence that is introduced in the host's genome by recombinant techniques. Said foreign DNA includes e.g. a T-DNA sequence or a part thereof such as the T-DNA sequence comprising the selectable marker in an expressible format. Foreign DNA furthermore include intervening DNA sequences as defined supra.

With "recombination event" is meant either a site-specific recombination event or a recombination event effected by transposon 'jumping'.

With "recombinase" is meant either a site-specific recombinase or a transposase.

With "recombination site" is meant either site-specific recombination sites or transposon border sequences.

With "site specific recombination event" is meant an event catalyzed by a system generally consisting of three elements: a pair of DNA sequences (the site-specific recombination sequences or sites) and a specific enzyme (the site-specific recombinase). The site-specific recombinase catalyzes a recombination reaction only  
5 between two site-specific recombination sequences depending on the orientation of the site-specific recombination sequences. Sequences intervening between two site-specific recombination sites will be inverted in the presence of the site-specific recombinase when the site-specific recombination sequences are oriented in opposite directions relative to one another (i.e. inverted repeats). If the site-specific recombination  
10 sequences are oriented in the same direction relative to one another (i.e. direct repeats), then any intervening sequences will be deleted upon interaction with the site-specific recombinase. Thus, if the site-specific recombination sequences are present as direct repeats at both ends of a foreign DNA sequence integrated into a eukaryotic genome, such integration of said sequences can subsequently be reversed by interaction of the  
15 site-specific recombination sequences with the corresponding site specific recombinase. A number of different site specific recombinase systems can be used including but not limited to the Cre/lox system of bacteriophage P1, the FLP/FRT system of yeast, the Gin recombinase of phage Mu, the Pin recombinase of *E. coli*, the PinB, PinD and PinF from *Shigella*, and the R/RS system of *Zygosaccharomyces rouxii*. Recombinases generally  
20 are integrases, resolvases or flippases. Also dual-specific recombinases can be used in conjunction with direct or indirect repeats of two different site-specific recombination sites corresponding to the dual-specific recombinase (Baszczynski et al. 1999 - WO9925840). The preferred site-specific recombinase systems are the bacteriophage P1 Cre/lox, the yeast FLP/FRT and the *Z. rouxii* R/RS systems. In these systems a  
25 recombinase (Cre, FLP or R) interact specifically with its respective site-specific recombination sequence (lox, FRT, or RS respectively) to invert or excise the intervening sequences. The site-specific recombination sequences for each of these two systems are relatively short (34 bp for lox and 47 bp for FRT). Some of these systems have already been used with high efficiency in plants such as tobacco (Dale et al. 1990, Onouchi et al. 1991, Sugita et al. 2000) and *Arabidopsis* (Osborne et al. 1995, Onouchi  
30 et al. 1995). Site-specific recombination systems have many applications in plant molecular biology including methods for control of homologous recombination (e.g. Hodges et al. 1996 - US5527695), for targeted insertion, gene stacking, etc.

(Baszczynski et al. 1999 - WO9925821) and for resolution of complex T-DNA integration patterns or for excision of a selectable marker (Ow et al. 1999 - WO9923202).

Although the site-specific recombination sequences must be linked to the ends of the DNA to be excised or to be inverted, the gene encoding the site specific recombinase  
5 may be located elsewhere. For example, the recombinase gene could already be present in the eukaryote's DNA or could be supplied by a later introduced DNA fragment either introduced directly into cells, through crossing or through cross-pollination. Alternatively, a substantially purified recombinase protein could be introduced directly into the eukaryotic cell, e.g. by micro-injection or particle bombardment. Typically, the  
10 site-specific recombinase coding region will be operably linked to regulatory sequences enabling expression of the site-specific recombinase in the eukaryotic cell.

With "recombination event effected by transposon jumping" or "transposase-mediated recombination" is meant a recombination event catalyzed by a system consisting of three elements: a pair of DNA sequences (the transposon border sequences) and a  
15 specific enzyme (the transposase). The transposase catalyzes a recombination reaction only between two transposon border sequences which are arranged as inverted repeats. A number of different transposon/transposase systems can be used including but not limited to the Ds/Ac system, the Spm system and the Mu system. These systems originate from corn but it has been shown that at least the Ds/Ac and the Spm system  
20 also function in other plants (Fedoroff et al. 1993, Schlappi et al. 1993, Van Sluys et al. 1987). Preferred are the Ds- and the Spm-type transposons which are delineated by 11 bp- and 13 bp- border sequences, respectively.

Although the transposon border sequences must be linked to the ends of the DNA to be excised, the gene encoding the transposase may be located elsewhere. For example,  
25 the recombinase gene could already be present in the eukaryote's DNA or could be supplied by a later introduced DNA fragment either introduced directly into cells, through crossing or through cross-pollination. Alternatively, a substantially purified transposase protein could be introduced directly into cells, e.g. by microinjection or by particle bombardment.

30 As part of the current invention, transposon border sequences are included in a foreign DNA sequence such that they lie outside said DNA sequence and transform said DNA into a transposon-like entity that can move by the action of a transposase.

As transposons often reintegrate at another locus of the host's genome, segregation of the progeny of the hosts in which the transposase was allowed to act might be necessary to separate transformed hosts containing e.g. only the transposon footprint and transformed hosts still containing the foreign DNA.

5 In performing the present invention, the genetic element is preferably induced to mobilize, such as, for example, by the expression of a recombinase protein in the cell which contacts the integration site of the genetic element and facilitates a recombination event therein, excising the genetic element completely, or alternatively, leaving a "footprint", generally of about 20 nucleotides in length or greater, at the original  
10 integration site. Those hosts and host parts that have been produced according to the inventive method can be identified by standard nucleic acid hybridization and/or amplification techniques to detect the presence of the mobilizable genetic element or a gene construct comprising the same. Alternatively, in the case of transformed host cells, tissues, and hosts wherein the mobilizable genetic element has been excised, it is  
15 possible to detect a footprint in the genome of the host which has been left following the excision event, using such techniques. As used herein, the term "footprint" shall be taken to refer to any derivative of a mobilizable genetic element or gene construct comprising the same as described herein which is produced by excision, deletion or other removal of the mobilizable genetic element from the genome of a cell transformed previously with  
20 said gene construct. A footprint generally comprises at least a single copy of the recombination loci or transposon used to promote excision. However, a footprint may comprise additional sequences derived from the gene construct, for example nucleotide sequences derived from the left border sequence, right border sequence, origin of replication, recombinase-encoding or transposase-encoding sequence if used, or other  
25 vector-derived nucleotide sequences. Accordingly, a footprint is identifiable according to the nucleotide sequence of the recombination locus or transposon of the gene construct used, such as, for example, a sequence of nucleotides corresponding or complementary to a *lox* site, *frt* site or RS site.

The term "cell cycle" means the cyclic biochemical and structural events associated with  
30 growth and with division of cells, and in particular with the regulation of the replication of DNA and mitosis. Cell cycle includes phases called: G0, Gap1 (G1), DNA synthesis (S), Gap2 (G2), and mitosis (M). Normally these four phases occur sequentially, however, the cell cycle also includes modified cycles wherein one or more phases are absent



resulting in modified cell cycle such as endomitosis, acytokinesis, polyploidy, polyteny, and endoreduplication.

The term "cell cycle interacting protein", "cell cycle protein" or "cell cycle control protein" as denoted herein means a protein which exerts control on or regulates or is required for the cell cycle or part thereof of a cell, tissue, organ or whole organism and/or DNA replication. It may also be capable of binding to, regulating or being regulated by cyclin dependent kinases or their subunits. The term also includes peptides, polypeptides, fragments, variant, homologs, alleles or precursors (eg preproteins or preproteins) thereof.

Cell cycle control proteins and their role in regulating the cell cycle of eukaryotic organisms are reviewed in detail by John (1981) and the contributing papers therein (Norbury & Nurse 1992; Nurse 1990; Ormrod & Francis 1993) and the contributing papers therein (Doerner et al. 1996) which are incorporated by reference. (Elledge 1996; Francis, Francis & Halford 1995; Hirt et al. 1991; Mironov et al. 1999)

The term "cell cycle control genes" refers to any gene or mutant thereof which exerts control on or are required for: chromosomal DNA synthesis and for mitosis (preprophase band, nuclear envelope, spindle formation, chromosome condensation, chromosome segregation, formation of new nuclei, formation of phragmoplast, duplication of microtubule-organizing center, etc) meiosis, cytokinesis, cell growth, endoreduplication, cell cycle control genes are also all genes exerting control on the above: homologs of CDKs, cyclins, E2Fs, Rb, CKI, Cks, and also any genes which interfere with the above, cyclin D, cdc25, Wee1, Nim1, MAP kinases, etc.

More specifically, cell cycle control genes are all genes involved in the control of entry and progression through S phase. They include, not exclusively, genes expressing "cell cycle control proteins" such as cyclin dependent kinases (CDK), cyclin dependent kinase inhibitors (CKI), D, E and A cyclins, E2F and DP transcription factors, pocket proteins, CDC7/DBF4 kinase, CDC6, MCM2-7, Orc proteins, cdc45, components of SCF ubiquitin ligase, PCNA, DNA-polymerase.

The term "cell cycle control protein" include cyclins A, B, C, D and E including CYCA1;1, CYCA2;1, CYCA3;1, CYCB1;1, CYCB1;2, CYC B2;2, CYCD1;1, CYCD2;1, CYCD3;1, and CYCD4;1 (Evans et al. 1983; Francis et al. 1998; Labbe et al. 1989; Murray & Kirschner 1989; Renaudin et al. 1996; Soni et al. 1995; Sorrell et al. 1999; Swenson, Farrell, & Ruderman 1986) cyclin dependent kinase inhibitor (CKI) proteins such as ICK1

- (Wang, Fowke, & Crosby 1997), FL39, FL66, FL67 (PCT/EP98/05895), Sic1, Far1, Rum1, p21, p27, p57, p16, p15, p18, p19 (Elledge 1996;Pines 1995), p14 and p14ARF; p13suc1 or CKS1At (De Veylder et al. 1997;Hayles & Nurse 1986) and nim-1 (Fantès 1989;Russell & Nurse 1986;Russell & Nurse 1987b;Russell & Nurse 1987a;Russell & Nurse 1986) homologues of Cdc2 such as Cdc2MsB (Hirt et al. 1993) CdcMs kinase (Bogre et al. 1997) cdc2 T14Y15 phosphatases such as Cdc25 protein phosphatase or p80cdc25 (Bell et al. 1993;Elledge 1996;Kumagai & Dunphy 1991;Russell et al. 1986) and Pyp3 (Elledge 1996) cdc2 protein kinase or p34cdc2 (Colasanti, Tyers, & Sundaresan 1991;Feiler & Jacobs 1990;Hirt et al. 1991;John, Sek, & Lee 1989;Lee & Nurse 1987;Nurse & Bissett 1981;Ormrod et al. 1993) cdc2a protein kinase (Hemerly et al. 1993) cdc2 T14Y15 kinases such as wee1 or p107wee1 (Elledge 1996;Russell et al. 1986;Russell et al. 1987b;Russell et al. 1987a;Sun et al. 1999) mik1 (Lundgren et al. 1991) and myt1 (Elledge 1996); cdc2 T161 kinases such as Cak and Civ (Elledge 1996); cdc2 T161 phosphatases such as Kap1 (Elledge 1996); cdc28 protein kinase or p34cdc28 (Nasmyth 1993;Reed, Hadwiger, & Lorincz 1985) p40MO15 (Fesquet et al. 1993;Poon et al. 1993) chk1 kinase (Zeng et al. 1998) cds1 kinase (Zeng et al. 1998) growth-associated H1 kinase (Labbe et al. 1989;Lake & Salzman 1972;Langan 1978;Zeng et al. 1998) MAP kinases described by (Binarova et al. 1998;Bögge et al. 1999;Calderini et al. 1998;Wilson et al. 1999).
- 20 Other cell cycle control proteins that are involved in cyclin D-mediated entry of cells into G1 from G0 include pRb (Huntley et al. 1998;Xie et al. 1996) E2F, RIP, MCM7 and potentially the pRb-like proteins p107 and p130.
- Other cell cycle control proteins that are involved in the formation of a pre-replicative complex at one or more origins of replication, such as, but not limited to, ORC, CDC6, CDC14, RPA and MCM proteins or in the regulation of formation of this pre-replicative complex, such as, but not limited to, the CDC7, DBF4 and MBF proteins.
- 25 For the present purpose, the term "cell cycle control protein" shall further be taken to include any one or more of those proteins that are involved in the turnover of any other cell cycle control protein, or in regulating the half-life of said other cell cycle control protein. The term "protein turnover" is to include all biochemical modifications of a protein leading to the physical or functional removal of said protein. Although not limited to these, examples of such modifications are phosphorylation, ubiquitination and proteolysis. Particularly preferred proteins which are involved in the proteolysis of one or
- 30

more of any other of the above-mentioned cell cycle control proteins include the yeast-derived and animal-derived proteins, Skp1, Skp2, Rub1, Cdc20, cullins, CDC23, CDC27, CDC16, and plant-derived homologues thereof (Cohen-Fix & Koshland 1997; Hochstrasser 1998; Krek 1998; Lisztwan et al. 1998) and Plesse et al in (Francis et al. 1998)).

For the present purpose, the term "cell cycle control genes" shall further be taken to include any one or more of those gene that are involved in the transcriptional regulation of cell cycle control gene expression such as transcription factors and upstream signal proteins. Additional cell cycle control genes are not excluded.

For the present purpose, the term "cell cycle control genes" shall further be taken to include any cell cycle control gene or mutant thereof, which is affected by environmental signals such as for instance stress, nutrients, pathogens, or by intrinsic signals such as the animal mitogens or the plant hormones (auxins, cytokinins, ethylene, gibberellic acid, abscisic acid and brassinosteroids).

The term "cell cycle progression" refers to the process of passing through the different cell cycle phases. The term "cell cycle progression rate" accordingly refers to the speed at which said cell cycle phases are run through or the time spans required to complete said cell cycle phases.

With "pathogen" is meant those organisms that have a negative effect on the physiological state of the plant or a part thereof. Some pathogens are for instance viruses, bacteria, fungi, and parasitic plants. with plant "pests" is meant the group of nematodes as well as insects, which are able to attack and to damage the plant.

"Plant cell" comprises any cell derived from any plant and existing in culture as a single cell, a group of cells or a callus. A plant cell may also be any cell in a developing or mature plant in culture or growing in nature.

"Plants" comprises all plants, including monocotyledonous and dicotyledonous plants.

With "yeast two-hybrid assay" is meant an assay that is based on the observation that many eukaryotic transcription factors comprise two domains, a DNA-binding domain (DB) and an activation domain (AD) which, when physically separated (i.e. disruption of the covalent linkage) do not effectuate target gene expression. Two proteins able to interact physically with one of said proteins fused to DB and the other of said proteins fused to AD will re-unite the DB and AD domains of the transcription factor resulting in target gene expression. The target gene in the yeast two-hybrid assay is usually a

reporter gene such as the  $\beta$ -galactosidase gene. Interaction between protein partners in the yeast two-hybrid assay can thus be quantified by measuring the activity of the reporter gene product (Bartel and Fields 1997). Alternatively, a mammalian two-hybrid system can be used which includes e.g. a chimeric green fluorescent protein encoding reporter gene (Shioda et al. 2000). Yet another alternative consists of a bacterial two-hybrid system using e.g. *HIS* as reporter gene (Joung et al. 2000).

The term "fragment of a sequence" or "part of a sequence" means a truncated sequence of the original sequence referred to. The truncated sequence (nucleic acid or protein sequence) can vary widely in length; the minimum size being a sequence of sufficient size to provide a sequence with at least a comparable function and/or activity or the original sequence referred to, while the maximum size is not critical. In some applications, the maximum size usually is not substantially greater than that required to provide the desired activity and/or function(s) of the original sequence. Typically, the truncated amino acid or nucleotide sequence will range from about 5 to about 60 amino acids in length. More typically, however, the sequence will be a maximum of about 50 amino acids in length, preferably a maximum of about 60 amino acids. It is usually desirable to select sequences of at least about 10, 12 or 15 amino acids or nucleotides, up to a maximum of about 20 or 25 amino acids or nucleotides.

Furthermore, folding simulations and computer redesign of structural motifs of the protein of the invention can be performed using appropriate computer programs (Olszewski et al. 1996, Hoffman et al. 1995). Computer modeling of protein folding can be used for the conformational and energetic analysis of detailed peptide and protein models (Monge et al. 1995, Renouf et al. 1995). In particular, the appropriate programs can be used for the identification of interactive sites of the LRR receptor-like kinase of the present invention by computer assisted searches for complementary peptide sequences (Fassina and Melli 1994). Further appropriate computer systems for the design of protein and peptides are described in the prior art, for example in Berry and Brenner (1994), Wodak (1987), Pabo and Suchanek (1986). The results obtained from the above-described computer analysis can be used for, e.g. the preparation of peptidomimetics of the protein of the invention or fragments thereof. Such pseudopeptide analogues of the natural amino acid sequence of the protein may very efficiently mimic the parent protein (Benkirane et al. 1996). For example, incorporation of easily available achiral  $\omega$ -amino acid residues into a protein of the invention or a

fragment thereof results in the substitution of amino bonds by polymethylene units of an aliphatic chain, thereby providing a convenient strategy for constructing a peptidomimetic (Banerjee et al. 1996). Superactive peptidomimetic analogues of small peptide hormones in other systems are described in the prior art (Zhang et al. 1996). Appropriate peptidomimetics of the protein of the present invention can also be identified by the synthesis of peptidomimetic combinatorial libraries through successive amine alkylation and testing the resulting compounds, e.g., for their binding, kinase inhibitory and/or immunological properties. Methods for the generation and use of peptidomimetic combinatorial libraries are described in the prior art, for example in Ostresh et al. (1996) and Dorner et al. (1996).

Furthermore, a three-dimensional and/or crystallographic structure of the protein of the invention can be used for the design of peptidomimetic inhibitors of the biological activity of the protein of the invention (Rose et al. 1996, Ruterber et al. 1996).

To align sequences we used the Clustal V method described in Higgins and Sharp (Higgins DG & Sharp PM, *Comput Appl Biosci.* 1989 Apr;5(2)151-3). The Clustal Method groups sequences into clusters by examining sequence distances between all pairs. Clusters are aligned as pairs then collectively as sequence groups to produce the overall alignment. After the multiple alignment is completed, a neighbor-joining method is employed to reconstruct phylogeny for the putative alignment.

#### 1. Residue weight table

The Clustal method uses weight tables to construct multiple alignments. Residue weight tables are used in scoring protein and nucleotide alignments so that slightly mismatched residues, such as Ile vs. Val amino acids or G vs. R nucleotides, are not scored the same as total mismatches.

If your sequences are protein, or a mixture of protein and DNA, choose between PAM250, PAM100, Structural and Identity.

1. iPAM250 is recommended for the Clustal method and is ideal for longer sequences or highly diverged sequences.
2. PAM100 is also used with the Clustal method and is most accurate for shorter sequences or sequences having little divergence.
3. iStructural is recommended for the Jotun Hein method and gives higher scores to residues that are chemically and spatially similar.

4. Identity may be used with Clustal or Jotun Hein methods, but is recommended only for the Clustal method. The table scores non-identities as zero, and identities as a default value of 10.

## 2. Method Parameters

*A. Pairwise alignment parameters may include K-tuple, Gap Penalty, Window and Diagonals Saved:*

1. Specify the K-tuple, expressed as the unit's length in nucleotides. Any stretch of K or more adjacent nucleotides that form an exact match in both sequences is a K-tuple. A lower K-tuple will find smaller matching regions; a higher value will find fewer, but better, matching regions of similarity.
2. Type in the Gap Penalty, the amount deducted from the score for each gap in the alignment. Gaps of different sizes carry the same penalty.
3. Specify the Window, the range the program searches for all possible or alternate K-tuples. An extremely high value may slow an alignment by considering subalignments far from the main diagonal.
4. Type in the number of Diagonals Saved. In the pairwise calculation, the best scoring diagonals for each pair in the matrix are saved and used in the clustering and scoring of ancestors.

*B. Multiple alignment parameters include Gap Penalty and Gap Length Penalty.*

1. Specify the Gap Penalty, the amount deducted from the score for each gap in the alignment. Gaps of different sizes carry the same penalty.
2. Specify the Gap Length Penalty, the value which will be deducted from the alignment score after first multiplying it by the number of gaps. Longer gaps have a greater penalty than shorter gaps.

Whenever in the current description reference is made to a "phylogenetic tree", this phylogenetic tree is used to view phylogenetic relationships compatible with multiple sequence alignments. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events.

The phylogenetic tree gives a first estimate of the relationships between homologous sequences. It is highly recommended that your data be evaluated using additional methods before final conclusions are made about evolutionary relationships.

You can switch the view between Balanced Branches (phenogram) or Unbalanced Branches (cladogram). A phenogram averages the distances between ancestors in the tree. Dotted lines indicate a negative branch length, which may be a byproduct of averaging. In the cladogram, branch distances correspond to sequence divergence.

The compounds to be obtained or identified in the methods of the invention can be compounds that are able to bind to any of the nucleic acids, peptides or proteins of the invention. Other interesting compounds to be identified are compounds that modulate the expression of the genes or the proteins of the invention in such a way that either the expression of said gene or protein is enhanced or decreased by the action of said compound. Alternatively the compound can exert his action by directly or indirectly enhancing or decreasing the activity of any of the proteins of the invention.

Said compound or plurality of compounds may be comprised in, for example, samples, e.g., cell extracts from, e.g., plants, animals or microorganisms. Furthermore, said compound(s) may be known in the art but hitherto not known to be capable of suppressing or activating cell cycle interacting proteins. The reaction mixture may be a cell free extract or may comprise a cell or tissue culture. Suitable set ups for the method of the invention are known to the person skilled in the art and are, for example, generally described in Alberts et al., *Molecular Biology of the Cell*, third edition (1994), in particular Chapter 17. The plurality of compounds may be, e.g., added to the reaction mixture, culture medium or injected into the cell.

If a sample containing a compound or a plurality of compounds is identified in the method of the invention, then it is either possible to isolate the compound from the original sample identified as containing the compound capable of acting as an agonist, or one can further subdivide the original sample, for example, if it consists of a plurality of different compounds, so as to reduce the number of different substances per sample and repeat the method with the subdivisions of the original sample. Depending on the complexity of the samples, the steps described above can be performed several times, preferably until the sample identified according to the method of the invention only comprises a limited number of or only one substance(s). Preferably said sample comprises substances or similar chemical and/or physical properties, and most

preferably said substances are identical. Preferably, the compound identified according to the above described method or its derivative is further formulated in a form suitable for the application in plant breeding or plant cell and tissue culture.

5 The invention, now being generally described, will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain aspects and embodiments of the present invention and are not intended to limit the invention.



## DESCRIPTION OF FIGURES

### Figure 1. Models for stem-cell maintenance in the root and shoot meristem.

- (a) In the root meristem, the quiescent center (QC) functions as an organizing center. Those cells in immediate contact with the QC are stem cells (dark shading), which are initials for all cell files: endodermis (end), cortex (cort), epidermis (epi), lateral root cap (lrc) and columella (col). (b) In the shoot meristem, WUS is functionally equivalent to the QC in the root meristem in maintaining the presence of stem cells (dark shading). Long-range signaling for stem cell maintenance is also postulated for ZLL, which is expressed in the vascular cells. CLV3 is expressed in the presumptive stem cells that the initials for the L1 (epidermis), L2 (subepidermal tissues, i.e. mesophyll cells) and L3 (subepidermal tissues, i.e. vascular cells) tissue layers (Schnittger, Schellmann, & Hulskamp 1999). CLV1 is found in the L3 tunica layer of the central zone.

- Figure 2. List of the degenerative primers used for RT-PCR of root-specific members of the LRR receptor kinase family.

### Figure 3. Differential RT-PCR on hypocotyl/cotyledon (C) and roottip (R) RNA.

- The PCR fragments obtained upon amplification of R and C cDNAs with degenerate primers are separated on agarose gel. DNA fragments of different size can be detected in the R<sub>22</sub> compared to C<sub>22</sub> PCRs.

M represents the 100 bp marker (Boehringer). C and R indicated the origin of the cDNA sample used in the PCR and the numbers below indicate the primer combination used, e.g. C<sub>23</sub> represents hypocotyl/cotyledon cDNA amplified with primers LRR2 and KIN3.

### Figure 4. RT - PCR analysis of *RCH1* expression.

- Total RNA was isolated from flower (F), hypocotyl/cotyledon (C) and roottips (R) and *RCH1* mRNA was amplified for 26 cycles with RT-PCR using the RCH1F and RCH1R primers. As a control for cDNA quality and quantity as well as relative expression level, ubiquitin (Ubi) mRNA was amplified for 20 cycles under the same conditions using the UBIF and UBIR primers.

**Figure 5. The nucleotide sequence of the RCH1 operon.**

This figure shows the nucleotide sequence of SEQ ID NO 1 that contains the genomic RCH1 clone.

**Figure 6. The nucleotide sequence of the RCH1 promoter region.**

This fragment of the genomic RCH1 clone is about 3498 base pairs and is identified as SEQ ID NO 2. Two putative ATG start codons of the RCH1 protein on position 3479-3481 and on position 3491-3493 could be identified and are represented in bold. The underlined sequence corresponds to SEQ ID NO 18.

It is also known that there is a gene in the reverse orientation in this promoter region. This gene's open reading frame starts at the position 1759-1757 with the ATG (bold), contains two introns, and ends at position 350-348 with a TGA codon (bold). A homology search of the protein sequence of this antisense gene in the RCH1prom against the protein database with the program BLASTP 2.0.8 (Altschul et al. 1997) produced a hypothetical protein (Accession number AC002392, clone T20K24.8) that is a putative anthranilate N-hydroxycinnamoyl/benzoyltransferase from *Arabidopsis thaliana*. Both sequences show 38% identity and 54% of their amino acids are homologous.

**Figure 7. Promoter activity of the RCH1 promoter – GUS fusion construct in the *Arabidopsis thaliana* roots.**

Seedlings of different age were grown and stained with Glucuronidase (GUS). The expression of the GUS reporter gene can be seen in the root meristem of the main and the lateral roots, as well as in the vascular tissue of the root and the lateral root primordia. (A) 1 day old seedling stained over night, (B) higher magnification of A, (C) 2 days old seedling stained for 5 hours, (D) main root from 1 day old seedling stained for 1 hour, (E) lateral root primordia from 1 week old seedling stained for 5 hours, (F, G, H) lateral roots from an 8 days old seedling stained for 5 hours.

**Figure 8. The nucleotide sequence of the RCH1 gene.**

This fragment of the genomic RCH1 clone is about 3960 base pairs long and is identified as SEQ ID NO 3. Two putative ATG start codons of the RCH1 protein on position 1-3 and on position 13-15 could be identified and are represented in bold. Also the TAA

stopcodon on position 3958-3960 is represented in bold. There is a putative intron estimated to range from position 2900 (CAG<sup>^</sup>GTA) to position 3452 (CAG<sup>^</sup>AAT) with the program <http://www.CBS.DTU.DK/services/NetGene2>. The putative splice-sites are underlined.

5

**Figure 9. The amino acid sequence of the open reading frame encoding RCH1 protein.**

Two putative start methionins of the RCH1 protein on position 1 and on position 4 could be identified. An ORF of 1135 amino acids is identified and represented in SEQ ID NO 4.

10 The ORF of 1131 amino acids and starting at the methionine on position 4 (M) corresponds with SEQ ID NO 19.

The boxed peptide RYKILGAAQGLAYLHHDCVPPIVH corresponds with SEQ ID NO 5.

15 **Figure 10. Amino acid alignment of the RCH1 protein *the Arabidopsis thaliana* CLV1 receptor kinase like protein.**

The amino acid sequences of the RCH1 and of CLV1 (Genbank accession number AAB58929) were aligned using the program GAP as described in example 3. Match display thresholds for the alignment(s) are: | = IDENTITY, : = 2, . = 1.

20 **Figure 11. Phylogenetic tree of CLV1 homologues.**

The novel RCH1 sequence was aligned with the CLV1 (Genbank accession number U96879.1), the known CLV1 homologues BRI1 (Genbank accession number AF017056), HAE (GenPept accession number S27756) and ER (Genbank accession number D83257) as well as with 4 other related proteins that were retrieved from the public database searched with the RCH1 sequence using the BlastP program. The multiple alignment and phylogenetic tree of said sequences were calculated with the Clustal V method as described in example 4. Based on the sequence, RCH1 is more homologue to CLV1 than other known CLV-homologues.

30 **Figure 12. Phylogenetic tree of LRR receptor like kinases**

The novel RCH1 sequence was aligned with CLV1, its homologues BRI, HAE, ER and other LRR receptor like kinases retrieved from the public database. Also plant disease resistance genes, which comprise a leucine-rich repeat and which are receptor-like were

included in the alignment (RPS5, RPS2, RPP8, RPP1, RPS4, N, RPP5, Meyers *et al.*, 1999, *Plant J.*, Nov20(3): 317-32). The multiple alignment and phylogenetic tree of said proteins were calculated with the Clustal V method as described in example 4. Because of the close proximity of RCH1 to CLV1 in the phylogenetic tree, there is more evolutionary relationship between RCH1 and *clavata*, than with other kinds of LRR receptor like proteins such as plant disease resistance genes.

**Figure 13. The nucleotide sequence of a minimal promoter sequence of RCH1**

This figure shows the nucleotide sequence of SEQ ID NO. 18 that contains a minimal promoter sequence of the RCH1 clone. This fragment of the genomic RCH1 clone is about 842 base pairs. This sequence is deducted from the large promoter region identified as SEQ ID NO 2 (see example 7). The minimal promoter region correlates with the base pairs ranging from -1 of the first ATG defined in SEQ ID NO. 2 to -842 of the first ATG defined in SEQ ID NO. 2.

**Figure 14. Outprint of GenBank accession number AB017061 on December 4, 2000.**

**Figure 15. RCH1prom::GUS expression in embryo's**

Arabidopsis embryo transformed with RCH1prom::GUS. Embryo's were stained with beta glucuronidase. The RCH1 promoter is active very early in plant development and is only active in the root in the embryonic phase (A). On a stronger magnification (B) it is visible that the root specific expression is limited to the meristematic zone of the embryonic root.

**Figure 16. RCH1::GFP expression in roots**

RCH1 promoter is strongly active in endodermis, cortex, epidermis, lateral root cap, in short: in the "division zone". The RCH1 promoter is also active but in a low manner, in the quiescent center and in the vascular tissue.

**Figure 17. Amino acid sequence of RCH1 homologues of Table 4**

## EXAMPLES

Unless stated otherwise in the Examples, all recombinant DNA techniques are performed according to protocols as described in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY or in Volumes 1 and 2 of Ausubel et al., (1994), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd. (UK) and Blackwell Scientific Publications (UK).

### Example 1: Isolation of the RCH1 operon from root tissue of *Arabidopsis thaliana*.

Plant organ development depends on the presence of distally positioned groups of continuously dividing cells, the shoot and root apical meristems. Laser ablation studies suggest that a balance between signals for proper differentiation and short range signals from the mitotically inactive quiescent centre (QC), required to keep cells less differentiated, is important to maintain the root meristem (van den Berg et al. 1995; van den Berg et al. 1997). In the shoot meristem a balance between the *WUSCHEL* and *CLAVATA (CLV)* genes has been implicated to play an essential role in regulation cell division and differentiation (Clark et al. 1993; Fletcher et al. 1999; Schoof et al. 2000). The *CLV1* gene encodes a leucine rich repeat (LRR) receptor kinase (Clark, Williams, & Meyerowitz 1997), a member of a large gene family (Lease, Ingham, & Walker 1998). Other members are *ERECTA (ER)* (Torii et al. 1996), *BRASSINOLIDE INSENSITIVE (BRI)* (Li et al. 1997) and *HAESA (HAE)* (Jinn et al. 2000), genes which have a function in plant development

We investigated whether root specific *CLV1* homologs exist using a differential RT-PCR based approach. PCR products were generated from root tip and hypocotyl/cotyledon cDNA pools using degenerate primers, resulting in the isolation of the root specific *ROOT CLAVATA HOMOLOG 1 (RCH1)*. The promoter region of this gene was translationally fused to  $\beta$ -glucoronidase-green fluorescent protein (*GUS/GFP*) conferring root specific expression of these reporter genes (see Example 2).

### Designing degenerate primers

To RT-PCR root specific members of the LRR receptor kinase gene family we compared the sequences of the *CLV1*, *ER* and *HAE* genes and their encoded proteins. The degenerate LRR primers were designed against the NxLxGxIP encoding region of the xLxxNxLxGxIPxxLxxLxxLxxL consensus for the LRR receptor-like kinases. Degenerate kinase primers were designed for different homologous regions of the kinase domain. The primers are listed in Figure 2.

### RNA Isolation

For each RNA isolation the roottips and the hypocotyl/cotyledon part including the shoot apical meristem of *Arabidopsis thaliana* (Col0) plants, 4 days after germination, were collected.

Total RNA was isolated (Pawlowski et al. 1994) and chromosomal DNA contamination was removed upon Dnase I (Promega, Madison, WI) treatment, following the instructions of the suppliers. The amount and quality of RNA was determined using spectrophotometer and agarose gel electrophoresis (data not shown).

### Differential RT-PCR

Reverse transcription (RT) was performed using 5 µg of root tip (R) and hypocotyl/cotyledon (C) total RNA in a 20 µl reaction volume with 0.5 µg oligodT<sub>12-18</sub> (Pharmacia, New Jersey, USA) and 200 U Superscript II (LifeTechnologies, Paisley, UK), according to the instructions of the supplier. Following first strand cDNA synthesis the cDNA samples were treated with RNase H (LifeTechnologies) as instructed by the supplier and subsequently diluted to a total volume of 100 µl with water.

Amplification reactions were performed with all degenerate LRR and KIN primer pairs (Figure 2). For the polymerase chain reaction (PCR) 2.5 µl of the R or C cDNA sample was amplified in a total volume of 50 µl reaction volume containing, 100 µM dNTPs, 100 ng LRR and KIN primer, 1 U Taq polymerase (Boehringer, Ingelheim, Germany) and its accompanying buffer. The cDNAs were amplified during 40 cycles (1 min at 94°C, 1 min at 45-55°C, 3 min at 72°C).

### Cloning and sequencing

The amplified R and C cDNAs were separated and compared with agarose gel electrophoresis (Figure 3). The root specific fragments, e.g. fragments present in R<sub>22</sub> and not in C<sub>22</sub>, were isolated from gel, cloned into the pGEM-T vector (Promega) and transformed into *E. coli* (strain DH5 $\alpha$ ), using standard cloning procedures.

Several PCR fragments of the same size but from different members of the LRR receptor kinase gene family may be generated using degenerate primer pairs. Therefore, 24 randomly picked colonies obtained after transformation were used in a "colony PCR". Aliquots of the amplified inserts were separated on agarose gel followed by Southern transfer to a Supercharge N+ membrane (Schleicher & Schuell, Dassel, Germany) to create a "colony blot". An aliquot of one insert was labeled with <sup>32</sup>P-dCTP and hybridized to the colony blot to determine the number of clones it represented in the pool of 24. This procedure was repeated with other labeled inserts until all clones were identified.

A single representative of each pool of clones was sequenced and the sequence was analyzed using the BLAST program. One of the clones sequenced was named *RCH1* and showed strong homology to plant LRR receptor kinases.

### RT-PCR

The relative expression levels of *RCH1* in root tip (R), hypocotyl/cotyledon (C) and flowers (F) were determined using RT-PCR. Primers specific for the *RCH1* sequence were designed (RCH1F and RCH1R, Figure 2). The PCR samples were analyzed with agarose gel electrophoresis. *Ubiquitin (Ubi)* mRNA was used as an internal control (Horvath et al. 1993). Polyubiquitin genes consist of multiple units (Callis et al. 1995) and the *Ubi* primers hybridize with the ends of each single unit.

Independently isolated total RNA was reverse transcribed to obtain cDNA and for the PCR reaction 2  $\mu$ l of the cDNA sample was amplified in a total volume of 50  $\mu$ l as described above for the differential RT-PCR. The *RCH1* and *Ubi* cDNAs were amplified during 26 or 20 cycles (30 sec at 94°C, 30 sec at 56°C, 30 sec at 72°C), respectively (Figure 4). *RCH1* expression was only observed in root tip (R).

### Example 2: Promoter isolation and promoter fusion construct.

To determine if the *RCH1* promoter region is sufficient to confer root specific expression

of a "gene of interest" we decided to fuse this promoter to the Glucuronidase/Green Fluorescent Protein (*GUS/GFP*) reporter gene. The *RCH1* clone was used to isolate a phage containing the corresponding genomic region from a  $\lambda$ GEM (Promega, Madison, WI) based genomic library. The  $\lambda$ -GEM11 phage containing an insert of about 14550  
5 nucleotides comprising the *Arabidopsis thaliana* *RCH1* promoter and *RCH1* gene and also the surrounding genomic sequences was deposited on October 25, 2000 at the BCCM-LMBP plasmid collection and was named "lambda phage *RCH1* genomic". This deposit was given the accession number LMBP 5582CB by the international depositary authority. A restriction map was constructed of the genomic clone and a 7 kb *Xho*I  
10 fragment, containing 3.5 kb promoter and part of the *RCH1* coding sequence, was subcloned into the expression vector pBS (Stratagene, La Jolla, CA). The 3.5 kb promoter region including the first amino acids of the putative *RCH1* ORF is set forth in Figure 5 and is identified as SEQ ID NO 2. This promoter region was amplified using the primers M13F and PRCH1R and Pfu Taq polymerase (Stratagene, La Jolla, CA) in the  
15 manufacturer specified conditions. The promoter region was subsequently cloned into the binary pCAMBIA3383Xb vector (Roberts et al. 2000) creating a translational fusion with the *GUS/GFP* reporter gene.

#### **GUS histochemical assay.**

20 *Arabidopsis thaliana* plants were transformed with this construct according to the vacuum transformation method (Bechtold & Pelletier 1998). Transformants were selected for phosphinothricin-tripeptide (PTT, Duchefa, NL) resistance.

Histochemical localization of  $\beta$ -Glucuronidase activity (GUS) was performed using the substrate 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc, Biosynth AG, Staad,  
25 Switzerland). X-gluc was dissolved in n-dimethyl-formamide and diluted to 0.5 mg/ml in 50 mM NaPO<sub>4</sub>, pH 7.2 with 0.1% Triton X-100. Oxidative dimerization of the produced indoxyl derivative was enhanced by adding the oxidation catalyst K<sup>+</sup> ferricyanide/ferrocyanide to a final concentration of 0.5 mM (0.5 mM K<sub>4</sub>Fe(CN)<sub>6</sub>·H<sub>2</sub>O/0.5 mM K<sub>3</sub>Fe(CN)<sub>6</sub>). The GUS reaction was incubated at 37°C over several time periods,  
30 and pictures were taken from the cloured plant-tissues (Figures 7 and 15). In Figure 7 the very specific expression pattern of the *RCH1* promoter is visualized by the GUS reporter gene activity. Persons skilled in the art will also agree to the fact that the expression level of the *RCH1* promoter is very high, since after one hour the saturation



**Example 5: Root-specific reporter gene expression in Rice mediated by the *Arabidopsis thaliana* RCH 1 promoter.**

*CLAVATA* homologs are found in many other plant species from both the groups of monocots and dicots. Therefore one can assume that also the promoter of RCH1 is functional in those other plant species, and possibly exerts the same specific expression-pattern. To investigate the feasibility of root meristem-specific activation of the *Arabidopsis thaliana* RCH1 promoter in monocots, the aforementioned promoter-reporter gene construct, wherein the GUS/GFP chimeric gene is operably linked to the RCH1 promoter, will be transformed to rice using the standard transformation procedures well known to the persons skilled in the art and outlined in the following paragraph. After several time periods ranging from 1 day to 1 or more weeks, the seedling will be checked for the expression of the GUS reporter gene. This will be done by growing the seedlings in organogenesis medium, and staining them with glucuronidase for several time periods ranging from 1 hour to 5 hours.

**15 *Agrobacterium*-mediated rice transformation**

The Promoter-GUS hybrid gene outlined in Example 2 can be transformed to *Agrobacterium tumefaciens* strain LBA4404 or C58 by means of electroporation and subsequently transformed bacterial cells can be selected on a solid agar medium containing the appropriate antibiotics.

For demonstration of root-specific GUS expression with the promoter of the current invention, 309 mature dry seeds of the rice japonica cultivars Nipponbare or Taipei are dehusked, sterilised and germinated on a medium containing 2,4-dichlorophenoxyacetic acid (2,4-D). After incubation in the dark for four weeks, embryogenic, scutellum-derived calli are excised and propagated on the same medium. Selected embryogenic callus is then co-cultivated with *Agrobacterium*. Co-cultivated callus is grown on 2,4-D-containing medium for 4 to 5 weeks in the dark in the presence of a suitable concentration of the appropriate selective agent. During this period, rapidly growing resistant callus islands develop. After transfer of this material to a medium with a reduced concentration of 2,4-D and incubation in the light, the embryogenic potential is released and shoots develop in the next four to five weeks. Shoots are excised from the callus and incubated for one week on an auxin-containing medium from which they can be transferred to the soil. Hardened shoots are grown under high humidity and short days in a phytotron. Seeds

level of staining is reached in the meristem of the main root from a 1 week old seedling (Figure 7 D).

RCH1::GFP expression in roots is shown in Figure 16.

### **Example 3: Alignment.**

Sequence alignment of one complete register sequence against a target sequence as in figure 10 was done using the program GAP of the GCG package. The algorithm of Needleman and Wunsch is applied here to find the alignment of two complete sequences (Needleman and Wunsch, JMB 48(3): 443-453, 1970). The used parameters during the alignment were Gap Weight: 8, Average Match: 2.912, Length Weight: 2, Average Mismatch: -2.003, Quality: 1388, Length: 1149, Ratio: 1.416, and Gaps: 25.

### **Example 4: Phylogenetic tree.**

For sequence comparison of proteins as in figure 11 and 12, the Clustal V method to align all the sequences was used (Higgins DG & Sharp PM, *Comput Appl Biosci.* 1989 Apr;5(2):151-3). The Clustal Method groups sequences into clusters by examining sequence distances between all pairs. Clusters are aligned as pairs then collectively as sequence groups to produce the overall alignment. After the multiple alignment is completed, a neighbor-joining method is employed to reconstruct phylogeny for the putative alignment.

The Clustal method uses weight tables to construct multiple alignments. Residue weight tables are used in scoring protein and nucleotide alignments so that slightly mismatched residues, such as Ile vs. Val amino acids or G vs. R nucleotides, are not scored the same as total mismatches. For the alignment and pylogenetic trees in figure 11 and 12 the PAM250 weight table was used. Other method parameters for pairwise alignment were K-tuple: 1, Gap Penalty: 3, Window: 5 and Diagonals: 5. Other method parameters for multiple alignment include Gap Penalty: 10 and Gap Length Penalty: 10.

To view phylogenetic relationships compatible with multiple sequence alignments a Phylogenetic Tree was calculated. The length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. The phylogenetic tree gives a first estimate of the relationship between homologous sequences. A cladogram presentation of the phylogenetic tree is shown in figures 11 and 12.

can be harvested three to five months after transplanting. The method yields single locus transformants at a rate of over 50 % (Chan et al. 1993; Hiei et al. 1994)

**Example 6: Expression of a cytokinin oxidase cDNA under the root-specific RCH1 promoter in transgenic Tobacco and Rice leads to increased root production.**

The AtCKX1 gene (located in the database with the accession number: AC002510, *Arabidopsis thaliana* chromosome II section 225 of 255 of the complete sequence, sequence from clone T32G6) can be cloned under the control of the RCH1 promoter of Arabidopsis, in a binary vector. Also the ZmCKX1 from maize (accession number AF044603, (Morris et al. 1999) can be operably linked to the RCH1 promoter a binary vector. These genes can be transfected into Tobacco or Rice plants, using agrobacterium-mediated gene transfer. The transgenic plants expressing the ATCKX1 or the ZmCKX1 specifically in roots are expected to show increased root production without negatively affecting shoot development and without inducing premature leaf senescence.

**Example 7. Minimal promoter sequence.**

The length of the RCH1 promoter region (SEQ ID NO 2), which confers to root specific expression patterns, can be reduced to a "minimal promoter" without altering the root specific expression patterns or other features of the promoter such as constitutive and strong activity. As an example, the length of the promoter region was reduced to a minimal promoter sequence ranging from the -1 base pair just before the start codon at position 3479-3481 of SEQ ID NO. 2 to -842 base pairs before said start codon. Said minimal promoter is represented in SEQ ID NO. 18. This minimal promoter was PCR amplified and translationally fused to the GUS reporter gene. With a GUS histochemical assay (example 2) the same expression pattern as the large promoter region was demonstrated (results not shown). The difference is that the sequence before the HindIII site at the beginning of the minimal promoter is not necessary for the root specific expression pattern.

**Example 8. RCH1 prom::GUS expression in embryo's.**

Siliques of Arabidopsis plants transformed with RCH1prom::GUS were opened at different stages of development and the embryo's were surgically taken out of the ovules and transferred to X-gluc staining solution. Embryo's were stained for 0.5h – 4h before pictures were taken using light microscopy, Fig 15 A and B are at 25X and 40X magnification respectively, stained for 0.5 h. Longer staining did not reveal expression outside the root promeristem region.

RCH1 is active very early: RCH1 expression was first observed in torpedo stage embryo's in the root promeristem and was maintained in this region during the later stages of development. This figure shows that as soon as a meristem is formed RCH1 is expressed and provides further evidence that RCH1 expression is correlated with the appearance and presence of a root meristem from the embryonic stage onwards.

**Example 9. Isolation of cDNA of other Arabidopsis RCH1 homologues****Table 4: % identity with RCH1**

	Database entry genomic DNA	Database entry protein	% sequence identity with RCH1 on aa level
<b>RCH1.2</b>	AB028621 + AP002037	BAB03091	59.8%
<b>RCH1.3</b>	AB011476	BAB09286	40.1%
<b>RCH1.4</b>	AL022223	CAA1826 or T05050	38.9%
<b>RCH1. 5</b>	AC015446	AAG12526	42.1%

To discover the exact function of RCH1, the inventors want to circumvent the redundancy of the gene.

AB028621 (see figure 12, phylogenetic tree) is determined to be RCH1.2 protein and is also used by the inventors for further experiments. Double knock-outs of RCH1 and RCH1.2 were made and also other closely related sequences (AB011476.pro,

T05050.pro and AC015446.pro) are used in knock out experiments or in antisense expression.

### **Ectopic expression of RCH1**

The expression of RCH1 in normal cells correlates with the zone of rapidly dividing cells and is involved in the maintenance of meristem cells and dividing cells. Improved activity of RCH1 in a host cell results in the maintenance of cell division in that host cell and thus works against the differentiation of that cell. On the contrary diminished RCH1 activity in a host cell results in a diminished cell division in that host cell and thus in the loss of the amount of dividing cells in the meristem. Particular examples to influence these processes by altering the expression level of the RCH1 gene are described in the following paragraphs:

#### ***Overexpression***

In order to obtain higher activity of the RCH1 protein in the host cell the coding region of the RCH1 gene is fused to a strong promoters that is inducible or constitutive and/or ubiquitous or tissue specific.

To influence the RCH1 activity in a particular set of host cells, the RCH1 coding region is cloned under control of other meristemic promoters such as the promoters of WUSCHEL, CLV1, CLV2, CLV3, STM. The obtained effect is the control of the expression of RCH1 in a particular area of the meristem or in a particular time period of the development of the plant. Also the promoters of other RCH genes (such as AB028621.pro, AB011476.pro, T05050.pro and AC015446.pro) are used for this purpose.

In a particular application of the present invention, the RCH1 gene and the RKN gene (= the rice homologue of RCH1 as described in WO/04761) are operationally linked to the root-specific promoter PYK10, a constitutive ubiquitin or GOS 2 promoter and Cdc2a promoter and expressed in Arabidopsis or in rice.

### ***Downregulation***

In order to obtain lower activity of the RCH1 protein in the host cell, the coding region of the RCH1 gene cloned in a sense and antisense orientation in order to form a hairpin that induces gene-silencing in the host cell.

5

### ***Co-expression***

Downregulation or upregulation of the RCH1 in combination with downregulation or upregulation of other genes e.g.:

#### With WUSCHEL:

10 In the shoot meristem a balance between the *WUSCHEL* and *CLAVATA (CLV)* genes has been implicated to play an essential role in regulation cell division and differentiation (Clark et al. 1993; Fletcher et al. 1999; Schoof et al. 2000). The whole meristem maintenance is controlled by these genes and they seem to have an opposite function keeping the balance between cell division and differentiation. Therefore, *WUSCHEL* or a  
15 root *WUSCHEL* homologue is a preferred partner to be co-expressed with RCH1.

In an application of the invention RCH1 is upregulated in combination with the downregulation of *WUSCHEL* in a root specific manner. The obtained effect of this co-expression is a better control on the meristem maintenance.

#### With other RCH1 family members

20 Possibly the function of RCH1 is redundant and other closely related genes can take over its function. Therefore the RCH1 gene ectopically expressed in combination with closely related genes in order to downregulate or upregulate the activity of the encoded protein. The RCH1 homologues used for co-expression are for example AB028621.pro, AB011476.pro, T05050.pro and AC015446 (see figure 12 and table 4). The obtained  
25 effect is a better effect as described for the ectopic expression of RCH1 alone.

#### With other Clavata homologues:

Clavata 1 is a receptor that is involved in a signal pathway probably through the binding of a ligand (e.g. Clavata 3). A particular useful application of the present invention is the co-expression of RCH1 with the root Clavata 3 homologue RCH3. The obtained effect is  
30 a better control on meristem maintenance.

**Example 10: Domain swapping**

The N-terminal LRR domain of RCH1 functions as an extracellular perception domain and a C-terminal kinase domain functions in an intracellular signal transduction pathway. Previously it was described by He et al. (Science 2000, 288(5475):2360-3: Perception of  
5 brassinosteroids by the extracellular domain of the receptor kinase BRI1) that such domains can be swapped with the domains of other proteins. Following this methodology in a particular application of the present invention, the domains of RCH1 are swapped with the domains of another protein. In one example the LRR domain of RCH1 is replaced by the LRR domain of BRI. The effect obtained is the activation of a different  
10 pathway, which is normally not activated by RCH1, because the kinase domain of RCH1 now responds to another external signal that normally is not recognized by RCH1.

It will be clear that the invention may be practiced otherwise than as particularly described in the forgoing description and examples. Numerous modifications and  
15 variations of the present invention are possible in light of the above teaching and, therefore, are within the scope of the appended claims.

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**CLAIMS**

1. An isolated nucleic acid comprising a transcriptional regulatory root promoter or a functional fragment of such a promoter and/or comprising a nucleic acid sequence encoding a novel LRR receptor-like kinase (protein) or an immunologically active and/or functional fragment of such a protein (kinase) selected from the group consisting of:
- 5 (a) a nucleic acid comprising the DNA sequence as given in any of SEQ ID NOs 1, 2, 3 or 18, or the complement thereof,
- 10 (b) a nucleic acid comprising the RNA sequences corresponding to any of SEQ ID NOs 1, 2, 3 or 18, or the complement thereof,
- (c) a nucleic acid specifically hybridizing with the nucleotide sequence as defined in (a) or (b),
- (d) a nucleic acid encoding a protein comprising the amino acid sequence as given in SEQ ID NO 4, SEQ ID NO 5 or SEQ ID NO 19,
- 15 (e) a nucleic acid which is degenerated, as a result of the genetic code, to a nucleotide sequence as defined in any of (a) to (d), and which sequence codes for a protein having the amino acid sequence as given in SEQ ID NO 4, SEQ ID NO 19,
- 20 (f) a nucleic acid which is diverging, due to the differences in codon usage between the organisms, to a nucleotide sequence as defined in any of (a) to (d), and which sequence codes for a protein having the amino acid sequence as given in SEQ ID NO 4, SEQ ID NO 19,
- 25 (g) a nucleic acid which is diverging, due to the differences between alleles, to a nucleotide sequence as defined in any of (a) to (d), and which sequence codes for a protein having the amino acid sequence as given in SEQ ID NO 4, SEQ ID NO 19,
- 30 (h) a nucleic acid encoding a fragment of a protein encoded by a DNA sequence as given in SEQ ID NO 1 or SEQ ID NO 3 or encoding a fragment of a protein encoded by a nucleic acid as defined in any one of (a) to (g), wherein said fragment comprises the sequence as represented in SEQ ID NO 5, and,

- (k) a nucleic acid encoding a protein as defined in SEQ ID NO 4, SEQ ID NO 19 or a nucleic acid as defined in any one of (a) to (h), said nucleic acid interrupted by intervening DNA,  
provided that said nucleic acid is not one of the nucleic acids as deposited under the  
5 GenBank Accession numbers AB017061, AV538507, AV520681 or AQ966419.
2. An isolated nucleic acid comprising a transcriptional regulatory root promoter comprising the sequence as given in any of SEQ ID NOs 1, 2 or 18.
3. An isolated nucleic acid comprising a transcriptional regulatory root promoter consisting of the sequence as given in SEQ ID NO 2 or 18, or a functional part of  
10 said sequence which is able to regulate gene expression in a root specific manner, provided that said nucleic acid is not one of the nucleic acids as deposited under the GenBank Accession numbers AV538507 or AV520681.
4. An isolated nucleic acid comprising a transcriptional regulatory root specific promoter comprising:
- 15 (a) at least a functional part of the DNA sequence as given in any of SEQ ID NO 1, 2 or 18, and,  
(b) a second transcriptional regulatory sequence, optionally a regulatory promoter sequence not normally exhibiting root-specificity.
5. An isolated nucleic acid according to claim 4 wherein said second transcriptional regulatory promoter sequence is the ubiquitin promoter.  
20
6. An isolated nucleic acid selected from the group consisting of:
- (a) a nucleic acid consisting of at least part of the DNA sequence as given in SEQ ID NO 1 or 3, or the complement thereof,
- (b) a nucleic acid encoding a protein as given in SEQ ID NO 4 or 19 or encoding a  
25 fragment of said protein, wherein said fragment comprises the sequence as represented in SEQ ID NO 5, and
- (c) a nucleic acid encoding a protein with an amino acid sequence which is at least 65 % identical to the protein as given in SEQ ID NO 4 or 9, wherein said amino acid sequence comprises the sequence as represented in SEQ ID NO 5,

- or a homologue or a derivative of said protein, or an immunologically active and/or functional fragment thereof.
16. A polypeptide as defined in claim 15 being a root CLAVATA homologue or a functional homologue thereof
- 5 17. An isolated polypeptide comprising the sequence represented in SEQ ID NO 5 encodable by a nucleic acid of any of claims 1 to 7, or a homologue or a derivative thereof, or an immunologically active and/or functional fragment thereof.
18. A method for producing a polypeptide according to any of claims 15 to 17 comprising culturing a host cell of claim 13 or 14 under conditions allowing the expression of the  
10 polypeptide and recovering the produced polypeptide from the culture.
19. An antibody specifically recognizing an LRR receptor-like kinase (protein) of any of claims 15 to 17 or recognizing immunologically active parts or specific epitopes thereof.
20. A method for the production of transgenic plants, plant cells or plant tissues  
15 comprising the introduction of a nucleic acid molecule according to any of claims 1 to 7 in an expressible format or a vector according to any of claims 10 to 12 in said plant, plant cell or plant tissue.
21. The method of claim 20 further comprising regenerating a plant from said plant cell.
22. A transgenic plant cell comprising a nucleic acid sequence of any of claims 1 to 7  
20 which is operably linked to regulatory elements allowing transcription and/or expression of said nucleic acid in plant cells or obtainable by a method of claim 20 or 21.
23. The transgenic plant cell of claim 22 wherein said nucleic acid of any of claims 1 to 7 is stably integrated into the genome of said plant cell.
- 25 24. A transgenic plant or plant tissue comprising plant cells of claim 22 or 23.
25. A harvestable part of a plant of claim 24.
26. The harvestable part of claim 25 which is selected from the group consisting of seeds, leaves, fruits, stem cultures, rhizomes, tubers and bulbs.
27. The progeny derived from any of the plants or plant parts of any of claims 24 to 26.

characterised in that said nucleic acid encodes a novel LRR receptor-like kinase protein or an immunologically active and/or functional fragment of such a protein, and further provided that said nucleic acid is not one of the nucleic acids as deposited under the GenBank Accession numbers AB017061 or AQ966419.

- 5 7. An isolated nucleic acid according to any of claims 1 to 6 which is DNA, cDNA, genomic DNA, synthetic DNA, or RNA wherein T is replaced by U.
8. A nucleic acid molecule of at least 15 nucleotides in length hybridizing specifically with a nucleic acid of any of claims 2 to 7.
9. A nucleic acid molecule of at least 15 nucleotides in length specifically amplifying a  
10 nucleic acid of any of claims 2 to 7.
10. A vector comprising a nucleic acid sequence according to any of claims 1 to 7.
11. A vector according to claim 10 which is an expression vector wherein said nucleic acid sequence encoding a novel LRR receptor-like kinase or an immunologically active and/or functional fragment thereof, is operably linked to one or more control  
15 sequences allowing the expression in prokaryotic and/or eukaryotic host cells.
12. A vector according to claim 10 wherein said vector comprises at least part of a nucleic acid according to any of claims 2 to 7 and wherein said transcriptional regulatory root promoter or functional parts thereof is operably linked to one or more genes of interest.
- 20 13. A host cell containing a nucleic acid molecule according to any of claim 1 to 7 or a vector according to any of claims 10 to 12.
14. The host cell according to claim 13, wherein said host cell is a bacterial, insect, fungal, plant or animal cell.
15. An isolated LRR receptor-like kinase comprising one of the polypeptides selected  
25 from the group consisting of:
  - (a) a polypeptide as given in SEQ ID NO 4 or SEQ ID NO 19,
  - (b) a polypeptide comprising the amino acid sequence as given in SEQ ID NO 5, and,
  - (c) a polypeptide encoded by a nucleic acid as given in SEQ ID NO 1 or 3,



28. A method for conferring root-specificity, root-meristem-specificity, root-vascular-tissue-specificity, root-endodermis-specificity, root-cortex-specificity, root-epidermis-specificity, root-quiescent-center-specificity, and/or abundant expression in root and/or early expression in root to other promoter sequences comprising the fusion at  
5 least a functional part of the DNA sequence as given in SEQ ID NO 1, 2 or 18 to a second transcriptional regulatory promoter sequence normally not exhibiting root-specificity.
29. A method for root-specific expression of a gene(s) of interest comprising operably linking of said gene(s) of interest to a transcriptional regulatory root-specific promoter  
10 as defined in any of claims 2 to 5.
30. A method for modifying cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology comprising the modification of expression in the meristem of the main and the lateral roots, in the vascular tissue of the root or in the lateral root primordia of a gene(s) of interest operably linked to a  
15 transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
31. A method for phytoremediation comprising the expression of a gene(s) of interest under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
32. A method for environmental remediation comprising the expression of a gene(s) of  
20 interest under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
33. A method for enhancing plant growth and crop yield comprising the expression of a gene(s) of interest under the control of a a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
- 25 34. A method for conferring enhanced resistance to pathogens which attack the belowground plant tissue comprising the expression of a gene(s) of interest under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
- 30 35. A method for enhancing freezing tolerance in plants comprising the expression of a gene(s) of interest under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.

36. A method for enhancing resistance of a plant to drought and/or high salt comprising the expression of a gene(s) of interest under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.

5 37. A method for the production of light-sensitive proteins comprising the expression of a gene encoding said light-sensitive protein under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.

38. A method for root specific gene silencing comprising the expression of an antisense nucleotide sequence to the gene of interest, a gene silencing construct or a ribozyme under the control of a transcriptional regulatory root-specific promoter as defined in  
10 any of claims 2 to 5.

39. A method for stimulating root meristem formation comprising the expression of a gene that influences the proliferation and/or differentiation of the root meristem under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.

15 40. A method for stimulating root meristem formation comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.

41. A method stimulating root meristem formation comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like  
20 kinase as defined in any of claims 15 to 17 operably linked to a plant-operable promoter sequence.

42. A method stimulating root meristem formation comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase under the control of a transcriptional regulatory root promoter as defined in  
25 any of claims 2 to 5.

43. A method for stimulating root meristem formation comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.

- 4.1 A method for root-meristem maintenance comprising the expression of a gene that influences the proliferation and/or differentiation of the root meristem under the control of a transcriptional regulatory root-specific promoter as defined in any of claims 2 to 5.
- 5 45 A method for root-meristem maintenance comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.
- 46 A method for root-meristem maintenance comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like  
10 kinase as defined in any of claims 15 to 17 operably linked to a plant-operable promoter.
- 47 A method for root-meristem maintenance comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase under the control of transcriptional regulatory root promoter as defined in any  
15 of claims 2 to 5.
- 48 A method for root-meristem maintenance comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.
- 20 49 A method for enhancing root formation and/or root growth comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.
- 50 A method for enhancing root formation and/or root growth comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR  
25 receptor-like kinase as defined in any of claims 15 to 17 under the control of a plant operable promoter sequence.
- 51 A method for enhancing root formation and/or root growth comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase under the control of a transcriptional regulatory root promoter as  
30 defined in any of claims 2 to 5.

52. A method for enhancing root-formation and/or root growth comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.

5 53. A method for enhancing overall growth and yield comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.

54. A method for enhancing overall growth and yield comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR  
10 receptor-like kinase as defined in any of claims 15 to 17 operably linked to a plant operable promoter sequence.

55. A method for enhancing overall growth and yield comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase under the control of a transcriptional regulatory root promoter as  
15 defined in any of claims 2 to 5.

56. A method for enhancing overall growth and yield comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.

20 57. A method for modifying cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology comprising the modification of expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.

58. A method for modifying cell fate and/or plant development and/or plant morphology  
25 and/or plant biochemistry and/or plant physiology comprising the modification of expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase as defined in any of claims 15 to 17 operably linked to a plant-operable promoter sequence.

59. A method for modifying cell fate and/or plant development and/or plant morphology  
30 and/or plant biochemistry and/or plant physiology comprising the modification of expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding

an LRR receptor-like kinase under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.

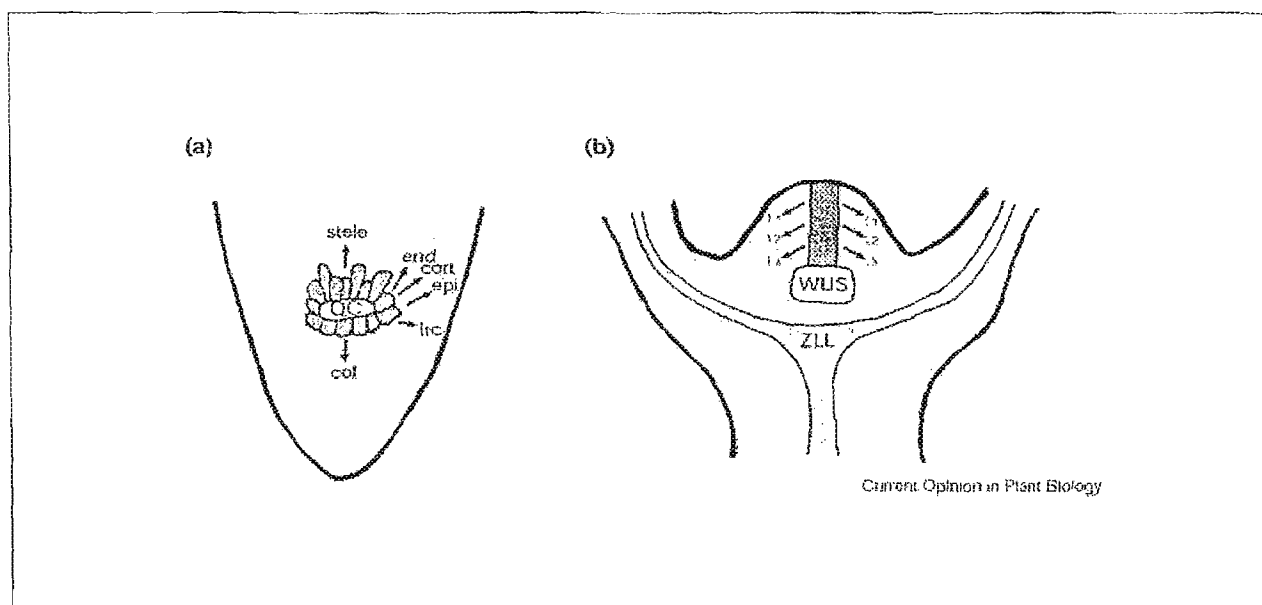
60. A method for modifying cell fate and/or plant development and/or plant morphology and/or plant biochemistry and/or plant physiology comprising the modification of  
5 expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.
61. A method to confer pathogen resistance to a plant comprising the expression in  
10 particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase operably linked to a plant-operable promoter sequence.
62. A method to confer pathogen resistance to a plant comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase as defined in any of claims 15 to 17 operably linked to a plant-operable promoter sequence.
- 15 63. A method to confer pathogen resistance to a plant comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding an LRR receptor-like kinase under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.
- 20 64. A method to confer pathogen resistance to a plant comprising the expression in particular cells, tissues or organs of a plant, of a nucleic acid encoding a polypeptide as defined in any of claims 15 to 17 under the control of a transcriptional regulatory root promoter as defined in any of claims 2 to 5.
65. A method for identifying and obtaining proteins interacting with a (LRR receptor-like kinase) polypeptide of any of claims 15 to 17 comprising a screening assay wherein  
25 a polypeptide of any of claims 15 to 17 is used or expressed.
66. A method according to claim 65 comprising a two-hybrid screening system wherein a nucleic acid encoding a polypeptide of any of claims 15 to 17 as a bait and a cDNA library as prey are expressed.

67. A method for modulating the interaction between an LRR receptor-like kinase (protein) as defined in any of claims 15 to 17 and interacting proteins obtainable by a method according to claim 65 or 66.
68. A method for identifying and obtaining compounds interacting with an LRR receptor-like kinase (protein) comprising the steps of:
- (a) providing a two-hybrid screening system wherein a polypeptide of any of claims 15 to 17 and a protein interacting with said LRR receptor-like kinase (protein) or an interacting protein obtainable by a method of claim 65 or 66 are expressed,
  - (b) interacting said compound with the complex formed by the expressed proteins as defined in a),
  - (c) detecting a second complex, wherein the presence of said second complex identifies a compound which specifically binds to one of said polypeptides or said second complex, and
  - (d) identifying the compound.
69. A method for identifying compounds or mixtures of compounds which specifically bind to a polypeptide of any of claims 15 to 17, comprising:
- (a) combining a polypeptide of any of claims 15 to 17 with said compound or mixtures of compounds under conditions suitable to allow complex formation, and,
  - (b) detecting complex formation, wherein the presence of a complex identifies a molecule which specifically binds said polypeptide.
70. Use of a molecule identifiable by means of a method of claim 68 or 69 as a plant growth regulator or herbicide.
71. A method for the production of a plant growth regulator or herbicide composition comprising the steps of the method of claim 68 or 69 and formulating the compounds obtained from said steps in a suitable form for the application in agriculture or plant cell or tissue culture
72. Use of a nucleic acid molecule of any of claims 1 to 7, a vector of claim 10 to 12, a polypeptide of claim 15 to 17 or an antibody of claim 19 for modifying cell fate and/or

plant development and/or plant morphology and/or plant biochemistry and/or plant physiology.

73. Diagnostic composition comprising at least one nucleic acid molecule of any of claims 1 to 7, vector of claim 10 to 12, polypeptide of claim 15 to 17 or antibody of claim 19.

Figure 1





## Primers used in experiments described

SEQ ID No.	Primer	Sequence (5'-3')	Details
SEQ ID No. 6	LRR1	AAYAAAYTTMASYGGTKMDWTHCC	Degenerate LRR primer
SEQ ID No. 7	LRR2	CARYTHAMCGGWGAAATHCC	Degenerate LRR primer
SEQ ID No. 8	LRR3	MAYYTSIYGGAHCTATHCC	Degenerate LRR primer
SEQ ID No. 9	KIN1	THCCWTTWGSCATGTANTCATA	Degenerate kinase primer
SEQ ID No. 10	KIN2	AYTCDGGDGCHAHGTADCC	Degenerate kinase primer
SEQ ID No. 11	KIN3	MAYHCCRAARCTRTAVACATC	Degenerate kinase primer
SEQ ID No. 12	RCH1F	CGATCAGACACAAGAACAT	<i>RCH1</i> specific primer
SEQ ID No. 13	RCH1R	AGCAATGGTGTGGAAGAA	<i>RCH1</i> specific primer
SEQ ID No. 14	UBIF	TGCAGATCTTYGTGAAGAC	Compl. to 5' end of repeating ubiquitin unit
SEQ ID No. 15	UBIR	GACTCCTTCTGGATGTTG	Compl. to 3' end of repeating ubiquitin unit
SEQ ID No. 16	M13F	TGTAAAACGACGGCCAGT	Compl. to pBS sequence
SEQ ID No. 17	PRCH1R	CACACAGGATCCAATCGGCATT GCAAAGACATA	Compl. to <i>RCH1</i> promoter, includes small coding sequence and <i>Bam</i> HI site

N=A+C+G+T; Y=C+T; M=A+C; S=C+G; K=T+G; W=A+T; R= A+G; D=A+T+G; H=A+T+C; V=A+C+G;  
I=deoxyinosine

Figure 3

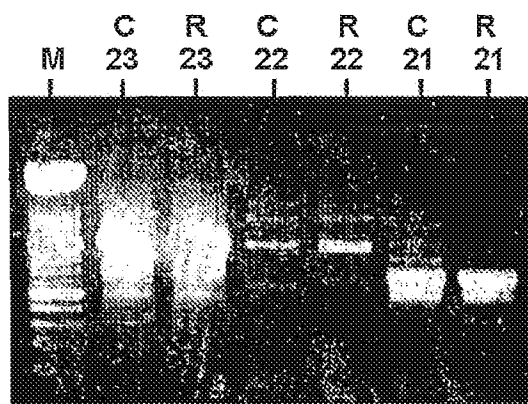


Figure 4

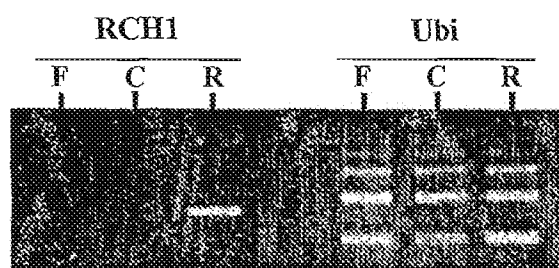


Figure 5-1 SEQ ID NO 1 : 7438 bp

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Figure 5-2

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GAGGTTGAAGAGATGATGCAACGCTAGGAGTCGCGCTTTTATGCATTAAATCCAAATACCGAAGACAGGCTACAATGAA  
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Figure 6 SEQ ID NO 2

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161    ACGGCATGTGTTATAGAACAACTTATCATCTGTATTAA CATAAAGCAAAAGTTCTTATACGACATAGTCCAAAGTTAC
241    AGACCATAAGCACACCTTATGATTTGTTGTACCTCTAAA ACACAGGATGATGACAAGAAGCAAAATTTGCCCAAAATTTAC
321    TGAGGGAAAAACGAGGCTTAACGCTCTCATATCTCAAAC AAAAAGCTTCTCABACAGTTTCATGTGTTTCAGATTGGAGGG
401    CAATGGCAACGGATAAGCTGCCATCATTAGTAGGACTTGG TAGCACAAGAGACAAACCTCGTATGGAATTCACCAGGT
481    CCCATAAAGATAGGACGACCCCAACCAAGTCTGCATCAT AAATAGGTAATCTAACCAGCTTGTGATTCCCAAATTTGG
561    GCACCTGTAGGTATGTGACCCGCGGACAAAGGCTGACAGA TCAGGCTGCATCTCCAGGTAGTCAAGAGCTGACCTCAGAT
641    AGTTATCATCCATACGAACCAAGAAATCATGAATCTGTCC TGCAGCATACCATGTTGGCTTAGATTAACAAATCCCTTGCA
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1281   AACATACATCATCAAGAATATCATTTACAGAACTAAAAAA CAGAACAACACAAGCATTCATTAATTAAGGAGGAAAG
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1441   GACGGAGATTAAGGCTAGGAGCAAAATCACCAGAAATCATC GATAACAGAAGGAGTATCAGCCACACGAAGAGAACACCA
1521   GCACCGTTACAACTCGATCTCAATACGACCATCATCGTCTC TCTTCAAGCGACCCAGCCATAGGGTAAAAAGGGACAGGGC
1601   TTTGGAAGAGCTTCCCTCATGACCTGAGGGTCAAGAGAAA TTGGAAGCGCGCGGTGGGTCTGTAGAAGTAGACACTAGGGG
1681   TATGGAATCTGGGGATGACAAGGTGACGCTTGGAGTTCCA AAGATTAGTGTATGGTCTCGGTGGCAGCGCCGACCATG
1761   GTGGAATCTCTGATGTTAATTTTCATATTTTAAAGAAAGA TCAAACGAACAACAATTTGTTTTGAGTTTGTATCTGAG
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1921   CCCACGACCCCGCTACTCCATCTGTGACCAACTCTCTC AGAAGGTTTATCATTGGTCCATCTCATCACTCGGTCTTAC
2001   TTTTCTATCCGGGGTTGGTAAACCCGTTCCCTCTGTCTTA AGGCCCAAATAATACTAATGGGCTTAAATAGTATAAAGTT
2081   CGAATGAAGAAGAGGAAAAAAATTTGACAAAGTTGATGTT TATAGCTACCGTACAAGTTAGTCAATCGAATGCCAAAGTT
2161   TGGCTTAACTTAACTCATTAACCGTAGCAGAGCATPAACA ACAACCACCAATAACATCTAACGGCGTAAACTCACGTGTT
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2481   ATAAAAATTCAATTTCTTTGAAGCATTCATTGGCTTAATG ATCCAAACCTTTTGTCTCTCCACTCTCTAAGAAAAATAAAG
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2881   AACAAATTTCTGAATGCAATATTTTCTTGTGTCATGAATAT TTAACTTGTATTAATTTCTTTTACTTTAGCTTAGGTGTGGACA
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Figure 7

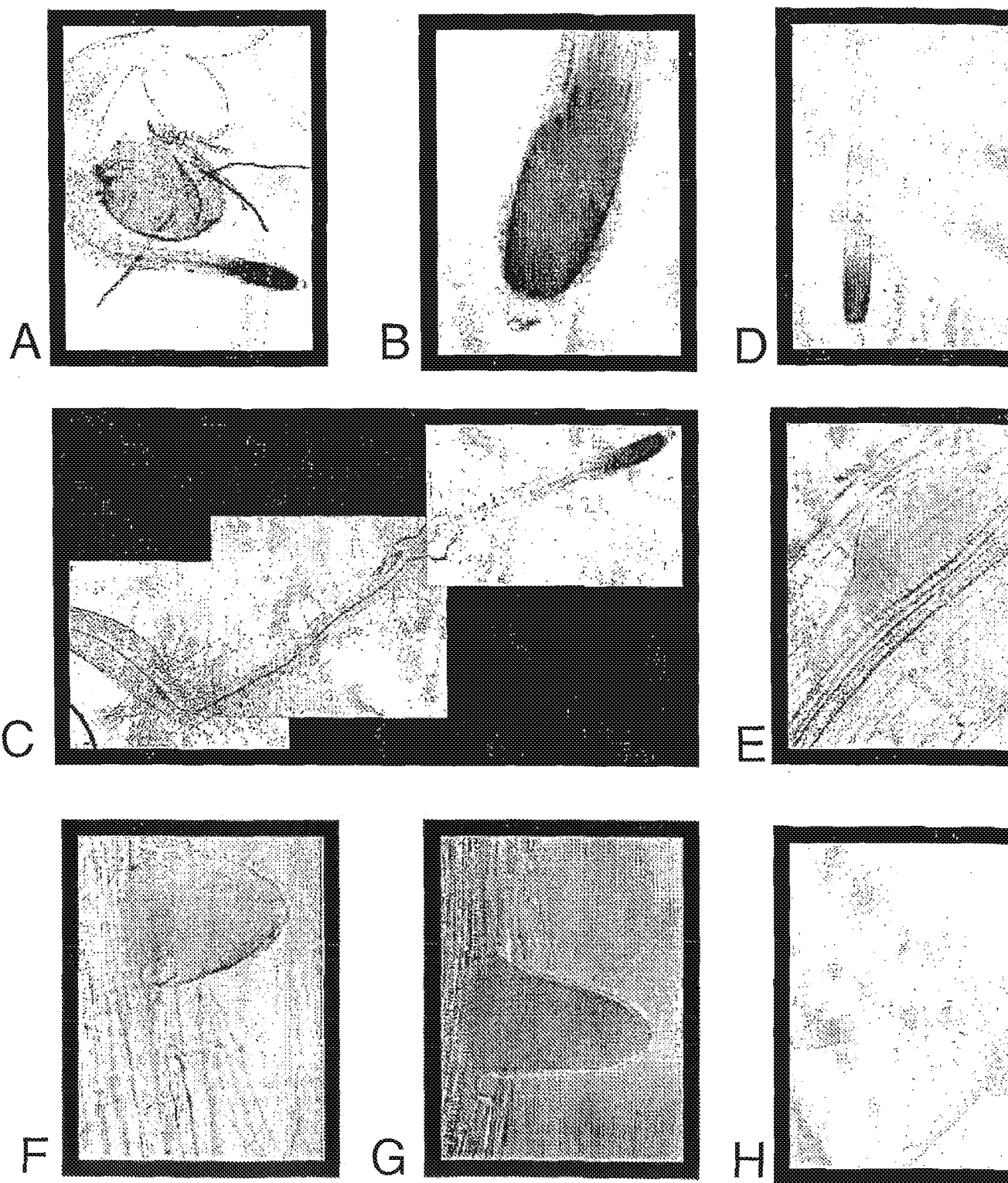


Figure 8 SEQ ID NO 3 : 3960 bp

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3 ACCGTCAGTCTTCTCCGGCTGGAATCCTTCTGATTCTGA CCCATGTCAATGGCCTTACATTACTTGTTCCTCCTCAGAC
4 ACACAACTCGTTACAGAGATCAATGTCGTCTCTGTTTCAGT TAGCTTTACCTTTCCCTCCTAACATT'TTCTCGTTTACTTC
5 A TTTAGAAAACGTTATCTCCAACTAATCTCACCGGA GCTATATCTTCTGAGATCGGAGATTGTTTCGGAGCTTATAG
6 CTATCGATTTAAGCTCAAATAGTCTTGTGGTGAAATACC TTCGAGTCTAGGGAAGCTCAAGAACTCTTCAAGAACTTTGT
7 TAAACTCCAATGGCCTCACAGGAAGATCCACCCGGAAC TCGCGGACTGCGTTAGCCTCAAGAACTCTTGAATTTTCGA
8 TAACTACTTATCCGAGAATCTCCCGTTGGAGCTCGGAAAG ATCTCGACTCTTGAAGAGCATAAGAGCCGGAGGAAACTCAG
9 ACGTTTCAGGGAAGATCCCGGAGGAGATCGGAAACTGTAG GAATCTCAAGGCTCTAGGGTTAGCAGCAACGAAATATATCC
10 GCTTCTTTACCTGTTTCTGTTGGGTCAACTAAGCAAGCTCC AGTCTTTATCTGTCTATTCTACAAATGCTCTCAGGTGAGAT
11 CTTCTAAAGAGCTTGGAACTGCTCTGAACCTATCAATCTG TTTCTATACGACAATGACTTGTCCGGCAGCTTCCAAAAG
12 AACTAGGAAAGCTTCAAACCTAGAGAAGATGCTTCTATG GCAGAAATATCTCCACGGACCTATCCCTGAGGAGATTGGA
13 TTCTATGAAAGCTTAAACGCCATTGATCTCTCTATGAATG ATTTCTCAGGAACCATCCCTAAATCGTTCGGAGCTTATGC
14 AATCTCCAAGAGCTTATGCTTAGCAGCAACAACATCACC GGGTCAATACCTTCGATTCTAAGTAAGTGCACAAAGCTTG
15 TTCAGTTCAGATTGACGCCAATCAGATTTCAGGTTTGAT TCCACCAGAGATTGGATTGCTTAAGGAGCTCAACATCTTC
16 TTXGGATGGCAGAATAAGCTAGAAGGAATATTCGGGACG AGTTAGCTGGTTGTGAGAACTCTTCAAGCTCTTGACTTGTG
17 ACAGAAATATCTCACCGGATCATTAACCTGAGGTTTGTTC CAGCTTCCGTAATCTGACTAAGCTTGTGCTCAAGTAATGC
18 AATCTCCGGTGTATCCCACTGGAGATCGGGAACCTGCAC TTGCTTGTGAGATTAAGGCTTGTCAATAACAGAAATCACC
19 AAGAAATCCCTAAAGGTATTGGATTCTTCAAGAACTTA GCTTCCCTCGACTTGTCTGAGAACAACTCTTCCCGTCCAGT
20 CTCTTTGGAGATAAGTAATTGTAGACAACTCCAGATGCTT AACTTGAGCAACAACACTCTTCAAGGTTATCTTCTCTGT
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22 CATCTCATTTCACTCAACCGGCTCATCTCAAGTAAGAATT CTTCAATGGAGAAATCCCTTCGTCTCTCGGTCACTGCAC
23 CAATCTTCAGCTTCTTGATCTCAGCAGTAATAACATCTCT GGAATATACCCGGAGGAGCTTTTGGACATCCAAGATCTAG
24 AATTTCGGTTAAACTTGAGCTTGAATTCATTAGATGGCTT TATCCCGGAAAGGATTTCCCGGCTTAACCGATTATCCGTG
25 CTGATATTTTCGCATAACATGCTTTCAGGCGACCTTCTG CGCTGTCCGGTCTAGAAAACCTTGGTTTCTTGAACATCTC
26 CTATAACAGATTCTCAGGTTATCTTCCAGATAGTAAAGTG TTTAGACAGCTGATAGGAGCAGAGATGGAAGGAAACATG
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28 AACTCAGGATAGCCATTGGATTGCTAATCAGCGTGACAG CGGTTCTAGCGGTATTAGGCGTGTGGCGGTTATACGAGC
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34 TACGAAAGGAGCGGTGTATGTAGCTTAGGATGGGAAGTGA GGTACAAGATTATACTTGGTGAGCTCAGGCTTTGGCTTA
35 CTTCGCCCATGACTGTGTTCTCCCATGTTTCATAGAGAC ATCAAGGCAAAATAATATTCTGATTGGCCCTGATTTTGAAC
36 CTACATTGGAGATTTCGGACTCGCTAAGCTTGTGATGA TGGCGACTTTGCTCTGTTCTTCCAACACCATTTGCTGGTTCC
37 TATGGTTACATAGCTCCAGGTACTTCCCAATTTCTGTTT TGCTGCTAATTAGTTAACTTGATACATCAAGTACTAG
38 TAGTCTTAGCCTAGTAATGGTAGAAATTCAGGTTTATAGT TTGGTTTGGTTT'TTGGCATTTTGGAAAGAAATAGTATTTT
39 GCTTCAGTTGATTTTCGATTGTTGTTTAAATTTTAGAAAA AAAATGGTAGAAGAAATAATTTTGTATTAATATTATATCA
40 CTACTGTATACGATTFAAAAAAAATTTGATTTGGTTTG GTCTCGATATTTTGTCTGTAGCTTGGTTCAATATTTTAC
41 TATATATATGATTCATGACTTTCATACTAATCAATTTTG TTCGTTTTCGTTTGTGATTAGTCTGAAAGTGAATCATATAG
42 TTAATAATATTGACCAATCTATCATATACTTACACGCTACA AGATTTCGATGTAATTTTGGGTGAAGCATTTTGGTTTTT
43 ATATGTTTCATCCTTAGTAACATAACAACAAATATCAATTGG GAAGCATAACGTTATATATTTCCCATAGGCTATAACACTA
44 TAATTATGTCAGAAATACGGATACTCAATGAAGATAACAGAG AAAAGCGACGTGTACAGCTATGGAGTCTGAGTGTCTGAGG
45 TACTAACGGGTAAGCAACCAATCGATCCAACGATACCAGA TGGACTCCACATAGTGGACTGGGTCAAGAAAATCAGAGAC
46 ATACAAGTAATCGACCAAGGACTACAAGCAAGACCGAGGT CAGAGGTTGAAGAGATGATGCAACGCTAGGAGTCTCGCT
47 TTTATGCATTATCCAATACCCGAAGACAGGCTACAATG AAAGATGTGGCTGCTATGCTTAGTGAGATATGTCAAGAGA
48 GAGAGGAATCGATGAAAGTTGATGGTTGCTCGGGAAGTTG TAACAATGGAAGAGAACGTGGCAAGATGATTCCGACTTCA
49 TCGGTTATGCAACAAACGGCTAAGTATTTGAGAAGTAGTA GCACAGATTCTCTGCGTCTTCTTGTCTTACTCTTCTTC
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```

Figure 9

```

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161    LNSNGLTGKIPPELGDCVSLKNLEIFDNYLSENLPLELCK  ISTLESTRAGGNSSELSGKIPEEIGNCRNLKVLGLAATKIS
241    GSLPVSLGQLSKLQSLFVYSTMLSGEIPKELGNCSELINL  FLYDNDLSGTLPKELGKLQNLEKMLLWQNNLHGPIPEEIG
321    FMKSLNAIDLSMNYFSGTIPKSFGNLSNLQELMLSSNNIT  GSIPSLSDCTKLIVQFQIDANQISGLIPPEIGLLKELNIF
401    LGWQNKLEGNIPDELACQNLQALDLSQNYLTGSLPAGLF  QLRNLTKLLLSNAISGVIPLETCNCTSLVRLRLVMNRIT
481    GEIPKGIGFLQNLSTFLDLSENNLGGPVPLEISNCRQLQML  NLSNNTLQGYLPLSLSSLTKLQVLDVSSNDLTGKIIPDSLQ
561    HLISLNRLLLSKNSFNGEIPSSLGHCNTNLQLDLSSNNIS  GTIPEELFDIQDLIALNLWNSLDGFIPIERISALNRLSV
641    LDISHNMLSGDLSALSGLNLVSLNISHNRFSGYLPDSKV  FRQLIGAEMEGNGLCSKGFRSCFVSNSSQLTQRGVHSH
721    RLRIAIGLLISVTAVLAVLGVLAIRAKQMIRDDNDSETG  ENLWTWQFTPPQKLNFTVEHVLKCLVEGNVIGKCGSGIVY
801    KAEMPNREVIHAVKKLWPVTVPNLNEKTKSSGVRDSFSAEV  KTLGSIRHKNIVRFLGCCWNKNTRLIMYDYSNGSLGSL
881    HERSGVCSLGWEVRYKTIILGAAOGKAYLHHDVPEIVHRD  IKANNILIGPDFEPYIGDFGLAKLVDDGDFARSSNTIAGS
961    YGYIAPEYGYSMKITEKSDVYSYGVVLEVLTKQPIDPT  IPDGLHIVDWVKKIRDIQVIDQGLQARPESEVEEMQTLG
1041   VALLCINPIPEDRPTMKDVAAMLSEICQEREESMKVDGCS  GSCNNGRERGKDDSTSSVMQQTAKYLRSSSTSFSSASSLLY
1121   SSSSSATSNVRPNLK

```



Figure 10-1

```

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      | | | | | | | | | | | | | | | | | |
51  NSPPPSVFGWNPSSDSDPCQWPYITCSSPDNKLVTETINVSVQLALPFPF 100
      | | | | | | | | | | | | | | | | | |
78  ...sftp.....lfgtispeigmthlvnltaannftgelpem 114
      ||| | | | | | | | | | | | | | | | |
101  NISSFTSLQKLVISNTNLTGAISSEIGDCSELIVIDLSSNSLVGEIPSSL 150
      | | | | | | | | | | | | | | | | | |
115  ksltslkvlnisnngnltgtfpgelkamvdlevldtynnnfngklppem 164
      | . | . | : . | | | | | | | | | | | | | | |
151  GKLKNLQELCLNSNG.LTGKIPPE.LGDCVSLKNLEIFDNYLSENPLEL 198
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165  selkklylsfggnf.fsgeipesygdigsleylglnaglsqkspafis 213
      . : | . : | | | | | | | | | | | | | | |
199  GKISTLESIRAGGNSELGKIPEEIGNCRNLKVLGLAATKISGSLPVSLG 248
      | | | | | | | | | | | | | | | | | |
214  rlknlremyigyynsytgvprefggltkleildmasctltgeiptsls 263
      . | | . : : | | | | | | | | | | | | | | |
249  QLSKLQSLFV.YSTMLSGEIPKELGNCSELINLFLYDNDLSGTLPKELGK 297
      | | | | | | | | | | | | | | | | | |
264  lkhltlflhinlthghippelsglvslksldlsinqltgeipqsfing 313
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      | | | | | | | | | | | | | | | | | |
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348  NLQELMLSSNNITGSIPSILSDCTKLQVQIDANQISGLIPPEIGLLKEL 397
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390  lilsnnfffgpipeelgkcksltkirivknllngtvpaglfnlplvtiie 439
      | : | . | | | | | | | | : : | | : : | | : :
448  LLLISNAISGVIPLETGNCTSLVRLRLVNNRITGEIPKGIGFLQNLFLD 497
      | | | | | | | | | | | | | | | | | |
440  ltdnffsgelpvtmsg.dvldqiylsnnwfsgeippaignfpnlqtlfld 488
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498  LSENNLSGPVPLEISNCRQLQMLNLSNNTLQGYLPLSLSSLTKLQVLDVS 547
      | | | | | | | | | | | | | | | | | |
489  rnrfgrnpreifelkhlsrintsannitggipdsisrcstlisvdlsrn 538
      | | | : | | | | | | | | | | | | | | |
548  SNDLTGKIPDSLGHLLISLNRLLSKNSFNGEIPSSLGHCTNLQLLDLSSN 597
      | | | | | | | | | | | | | | | | | |
539  ringeipkginnvknlg.tlnisgnqltgsiptgignmtslttldlsfnd 587
      | . | | . : . . . | | : | | | | | | : | . | : |
598  NISGTIPEELFDIQDLIALNLSWNSLDGFIPERISALNRLSVLDISHNM 647

```

Figure 10-2

```

588 lsgrv.plgg.....qf.....lvfne...tsfagntylcl 614
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    . . . . .
615 phrvsc....ptrpgqtsdhnhtalfspsriv.itviaaitglilisva 658
    || | | | | | | | | | | | | | | | | | | | | | |
698 KGFRSCFVSNSSQLTTQRGVHSHRLRIAIGLLISVTAVLAVLGVLAIRA 747
    . . . . .
659 ...irqmknkkngksl.awkltafqkldfksedvleclkeeniigkggag 704
    || | | | | | | | | | | | | | | | | | | | | | |
748 QQMIRDDNDSETGENLWTWQFTPQKLNFTVEHVLKCLVEGNVIGKCSG 797
    . . . . .
705 ivyrgsmpnnvdvaikrl.....vgrgtgrsd..hgftaeigtgrir 745
    |||: ||| :|:|:| . | | | | | | | | | | | |
798 IVYKAEMPNREVIHAVKKLWFPVTPNLNEKTKSSGVRDSFSAEVKTLGSIR 847
    . . . . .
746 hrhivrllygvankdtnlllyeympngslgellhgskg.ghlqwetrhrv 794
    |:.||| || | | | | | | | | | | | | | | | | | | | | | |
848 HKNIVRFLGCCWNKNTRLLMYDMSNGSLGSLHERSGVCSLGWEVRYKI 897
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795 aveaaaglcylhhdcspililhrdvksnilldsdfeahvadfglakflvd 844
    . |||.|| | | | | | | | | | | | | | | | | | | | | | |
898 ILGAAQGLAYLHDCVPPIVHRDIKANNILIGPDFEPYIGDFGLAKLVDD 947
    . . . . .
845 gaasecmssiagsygyiapeyaytlkvdeksdvysfgvvleliagkqp 894
    | . . . | | | | | | | | | | | | | | | | | | | | | | |
948 GDFARSSNTIAGSYGYIAPEYGYSMKITEKSDVYSYGVVVLEVLTKGQPI 997
    . . . . .
895 g.efgegvdivrwrnteeeitqpsdaaivvaivdprltgypltsv...i 940
    :|. || | | : : : : : : | | | | | | | | | | | |
998 DPTIPDGLHIVDWVKKIRD.....IQVIDQGLQARPESEVEEMM 1036
    . . . . .
941 hvfkiammcveeeaaarptmrevvhmltnppksvanliaf..... 980
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Figure 11

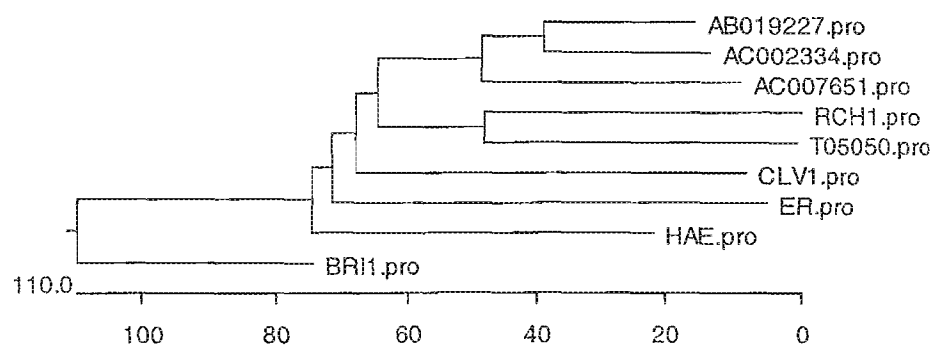
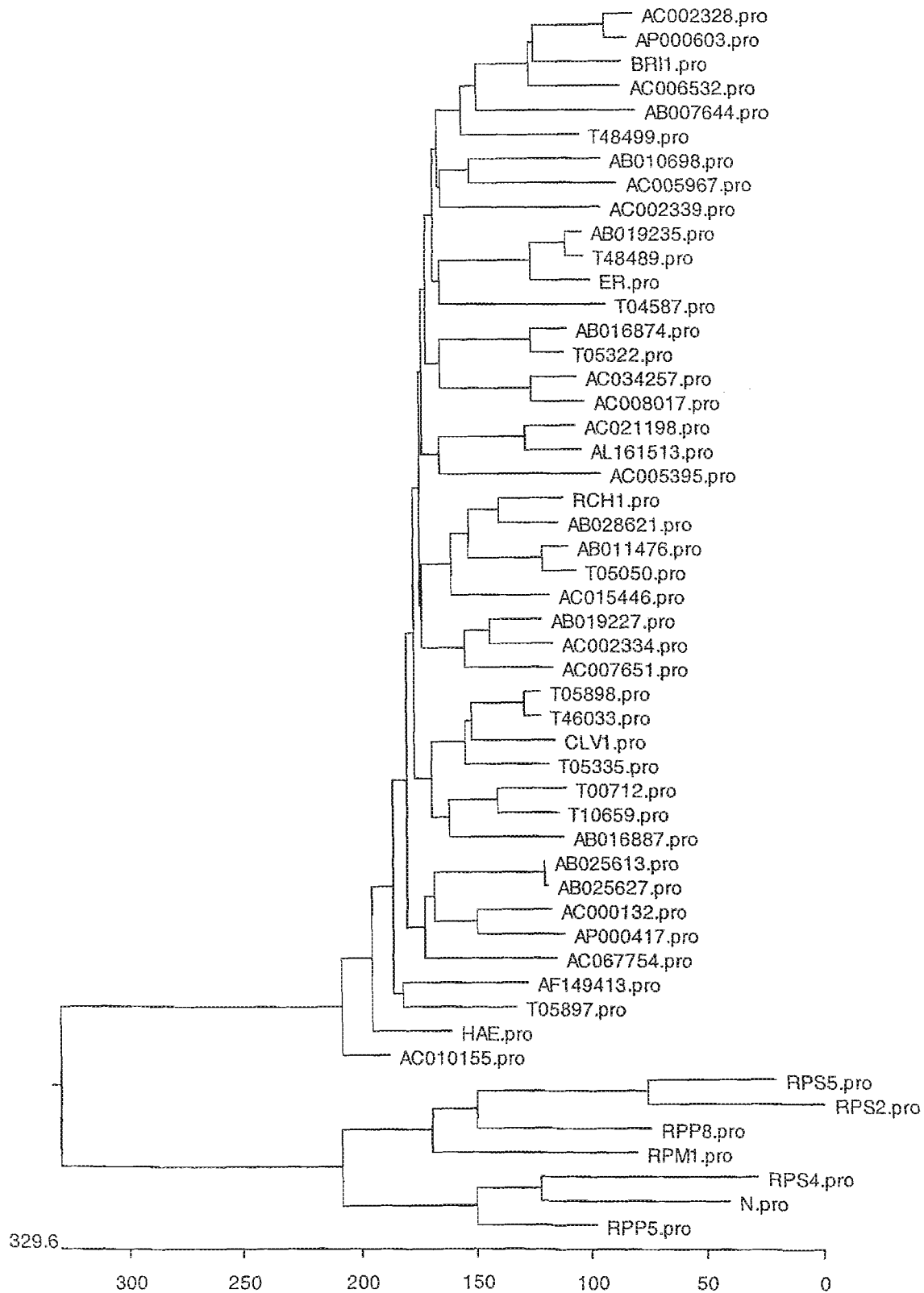


Figure 12



**Figure 13** SEQ ID NO 18 : 842 bp

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CTGAATGCAATATTTTCCTTGTCTGAATATTTAACTTGTTATTACTTTCTTTTAGCTTAGGTG  
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AACCAATGCCTTTTGTTTTGTGATATTTTATGTATATATAACTATTGACTTTTGTCTATGGA  
TAGTGTCTCGGGCTCTTGGCAAACATATTTCAAAGAAAAGTTAATGACTGTAATTAATTAATC  
TGAAGCTAGAAACAGAACCCCGAGGTAAAAGAAAAAGACAGAGCACATGAAGTTTAGTACTT  
TTATATATTTAATATATCATTCTTTCTTATTGCTTATCTCTAAAGCAAAAACCTCCCTAAACCT  
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GCAAAGAAAAAACTCTT

## NCBI Sequence Viewer

Figure 14

☒ NCBI
 ☒ Nucleotide banner

PubMed Nucleotide Protein Genome Structure PopSet Taxonomy OMIM

Search  for

View as    ☐ Hide Brief

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☒ 1: GI "3510337" [GenBank] Arabidopsis thaliana genom... PubMed, Protein, Related Sequences, 1

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 ACCESSION AB017061  
 VERSION AB017061.1 GI:3510337  
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 ORGANISM Arabidopsis thaliana  
 Eukaryota; Viridiplantae; Embryophyta; Tracheophyta; Spermatophyta; Magnoliophyta; eudicotyledons; core eudicots; Rosidae; eurosids II; Brassicales; Brassicaceae; Arabidopsis.  
 REFERENCE 1 (sites)  
 AUTHORS Kaneko,T., Katoh,T., Sato,S., Nakamura,Y., Asamizu,E., Kotani,H., Miyajima,N. and Tabata,S.  
 TITLE Structural analysis of Arabidopsis thaliana chromosome 5. IX. Sequence features of the regions of 1,011,550 bp covered by seventeen P1 and TAC clones  
 JOURNAL DNA Res. 6 (3), 183-195 (1999)  
 MEDLINE 99397451  
 REFERENCE 2 (bases 1 to 61712)  
 AUTHORS Nakamura,Y.  
 TITLE Direct Submission  
 JOURNAL Submitted (26-AUG-1998) to the DDBJ/EMBL/GenBank databases. Yasukazu Nakamura, Kazusa DNA Research Institute, Department of Plant Gene Research; 1532-3, Yana, Kisarazu, Chiba 292-0812, Japan (E-mail:ynakamu@kazusa.or.jp, Tel:81-438-52-3935, Fax:81-438-52-3934)  
 COMMENT Address for correspondence: kaos@kazusa.or.jp  
 For the latest information on annotation of this clone, please see [http://www.kazusa.or.jp/kaos/cgi-bin/agd\\_graph.cgi?c=K19E20](http://www.kazusa.or.jp/kaos/cgi-bin/agd_graph.cgi?c=K19E20)  
 Genes with similarity to proteins in the databases are described in 'product' or 'note' qualifiers. Genes that have no significant protein similarity are described as 'unknown protein'.  
 The software programs used to predict genes include: Grail (Informatics Group, Oak Ridge National Laboratory, <http://compbio.ornl.gov/Grail-1.3/>), GENSCAN (Chris Burge, MIT, <http://CCR-081.mit.edu/GENSCAN.html>), NetGene2 (S.M. Hebsgaard, et al., CBS, Technical University of Denmark, <http://www.cbs.dtu.dk/services/NetGene2/>) and SplicePredictor (Volker Brendel, Stanford University, <http://gremlin1.zool.iastate.edu/cgi-bin/sp.cgi>).  
 Genes encoding tRNAs are predicted by tRNAscan-SE (Sean Eddy, Washington University School of Medicine, St. Louis, <http://genome.wustl.edu/eddy/tRNAscan-SE/>).  
 This sequence may not be the entire insert of this clone. It may be shorter because we remove overlaps between neighboring submissions. The 5' clone is K24G6 and the 3' clone is K20J1.  
 FEATURES Location/Qualifiers

## NCBI Sequence Viewer

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CDS

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CDS



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CDS

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CDS

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## NCBI Sequence Viewer

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961 tttttgtgta atttgtctct tgtcgatca cctacatttt cagcattgga tgttataaat
1021 gcgaacaaac tataatgtaa aaaacatgtc agcagcatcg ggccatttca aactatgtaa
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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

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## NCBI Sequence Viewer

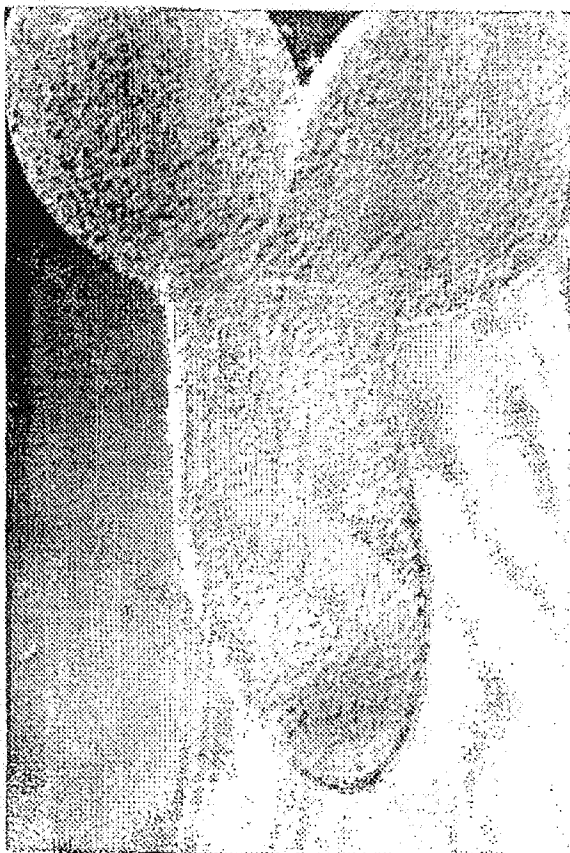
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FIGURE 15



**A**



**B**

FIGURE 16



## FIGURE 17

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Thr Ser Ala Ser Thr Asn Glu Val Ser Ala Leu Ile Ser Trp Leu His  
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Ser Ser Asn Ser Pro Pro Ser Val Phe Ser Gly Trp Asn Pro Ser  
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crop011pct.ST25.txt

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90

95

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Glu Ile Pro Ser Ser Leu Gly Lys Leu Lys Asn Leu Gln Glu Leu Cys  
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Cys Val Ser Leu Lys Asn Leu Glu Ile Phe Asp Asn Tyr Leu Ser Glu  
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Phe Val Tyr Ser Thr Met Leu Ser Gly Glu Ile Pro Lys Glu Leu Gly  
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crop011pct.ST25.txt

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crop01lpct.ST25.txt

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Gly Tyr Leu Pro Asp Ser Lys Val Phe Arg Gln Leu Ile Gly Ala Glu  
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crop01lpct.ST25.txt

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 Page 13

crop011pct.ST25.txt

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1090

1095

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crop011pct.ST25.txt

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crop011pct.ST25.txt

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crop011pct.ST25.txt

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crop011pct.ST25.txt

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crop011pct.ST25.txt

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crop011pct.ST25.txt

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1015

1020

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Leu Lys  
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